Microstructures, Rheological and Mechanical Properties of Gelatin–Starch Blends

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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February 2018
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2016
Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

Nuozi Zhang

6 February 2018
Acknowledgements

There are a large number of people to whom I wish to express my heartfelt gratitude, without them my PhD candidature would have been impossible.

Thank you to my supervisor Prof. Robert Shanks. You made my PhD experience interesting and enjoyable, and provided a good balance of support and independence. Your constant support and endorsement gave me confidence and purpose.

Dr Long Yu, you have been a fantastic supervisor. Your efforts, guidance, enthusiasm, practical advice and encouragements were continuous and paramount. Thank you for the valuable commercial experience and access to equipment I would not have otherwise been able to use. I look forward to working with you in the future.

Eustathios Petinakis, thank you for your friendship, advice and for listening. I know I can talk too much, but you never turned me away or failed to care. I appreciate all of your support, humour and effort. You were always my first port of call when things went wrong and I value your honesty, integrity, trust and creativity. I don’t think my PhD would have been any fun without you.

Pawen Swan, thank you for all the hospitality and support. You were always available to answer a question, have a laugh and offer help. You gave a great amount of input and valuable advice, all of which helped me throughout my PhD. A special thank you for taking me to conferences and for all the dinners! You kept my PhD fun and I can’t thank you enough.

Xiaoqing Zhang, thank you for giving me confidence and a passion for research during my Honours year. All of your lessons in crystallography were remembered, appreciated and used. Thanks for the DSLR loans!

Qiang Yuan, thank you for tirelessly repairing the X-ray equipment for me and patiently teaching me about X-ray instrumentation. I would also like to acknowledge and express my gratitude to the following University staff that have helped in numerous ways, especially Mrs. Zara Homan, Mrs. Nadia Zakhartchouk, Ms Ruth Cepriano, Mr. Sunly Prum, Ms. Robyne Drysdale, Mr. Karl Lang, and Mrs Dianne Mileo.

I would like to thank my friends and colleagues;

Micheal and Liang Zhang for your continuing support, friendship and encouragement.

Dongling Qiao, having you work alongside me for 6 months was a highlight of my PhD. Thank you for your friendship and contributions to my research.
Chengcheng Gao, thanks for keeping me fit! It’s been fantastic to have someone to share a break and physical exercise with and who listens to my rants about TV shows. Your friendship is dear to me and your opinions and guidance always helpful.

Finally I would like to thank my family, who understand the personal sacrifices required during a PhD.

Andy Yu and Di Pei, your support and warmth have never wavered. You welcomed me into your family and have always been compassionate and encouraging. I appreciate all your generosity and good will.

My partner Dr Penny Li, how can words express all my heartfelt thanks? You have been my guiding light, best friend and greatest supporter. Thank you for always patiently listening to my problems or doubts and offering a solution or suggestion. Your enduring love and support without any complaint or regret enabled me to complete this PhD. Thank you for lending me your strength, without you I would not have achieved my aspirations.

Finally, to my mother and father Wending Zhang and Ling Yu, you have both given me nothing but support. It’s been a long road from high school to PhD, and I know that I wasn’t fun to be around during exam times. Thank you for the encouragement, patience and understanding you have shown time and time again. You have always let me know that you are proud of me, which has motivated me to do my best. Your love and wisdom isn’t forgotten and I promise not wasted. This thesis is dedicated to you.
Publications from this Research

Journal Publications


Conference Papers


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List of Abbreviations

ATR-FTIR attenuated total reflection Fourier transform infrared spectroscopy

CSIRO Commonwealth Scientific and Industrial Research Organisation

CLSM confocal laser scanning microscopy

DMA dynamic mechanical analysis

DMTA dynamic mechanical thermal analysis

DSC differential scanning calorimetry

DTG derivative thermogravimetry curve

FTIR Fourier transform infrared

HPMC hydroxypropyl methylcellulose

HPS hydroxypropyl starch

IR infrared

MS molar substitution

NaOH sodium hydroxide

OM optical microscopy

PEG poly(ethylene glycol)

PET poly (ethylene terephthalate)

PLA poly(lactic acid)

PVC polyvinyl chloride

PVOH poly(vinyl alcohol)
RH - relative humidity
SAXS - small-angle X-ray scattering
SEM - scanning electron microscopy
TGA - thermogravimetry
TPS - thermoplastic starch
WAXS - wide angle X-ray Scattering

Young’s Modulus - tensile modulus describing the stiffness or elasticity of a material
XPS - X-ray photoelectron microscopy
XRD - X-ray diffraction
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Summary

Gelatin exhibits good film-forming and gas barrier properties, and it has been used widely in the food and pharmaceutical industries. However, the shortcomings of gelatin films, such as being an animal-derived ingredient, lower softening temperature and the instability of moisture content in gelatin, have led to attempts to use replacement substances. Starch is a common food ingredient and it has a facile film-forming behavior. Both gelatin and starch have separately been widely used to develop edible films. Therefore, development of starch–gelatin based blends and overcoming the shortcomings of gelatin-only products has both scientific and commercial importance.

Blends of gelatin with up to 50% hydroxypropylated high amylose content (80%) corn starch was developed for capsule materials. Poly(ethylene glycol) (PEG) was used as both a plasticizer and a compatibilizer in the blends. To prepare hard capsules for pharmaceutical applications using the well-established method of dipping stainless steel mold pins into solution, solutions with higher solids concentrations (up to 30%·w/w) were developed. The solutions, films and capsules of different gelatin–starch blends were characterized by viscosity, transparency, tensile testing, water contact angle and scanning electron microscopy (SEM). The linear microstructure of the high amylose starch, and the flexible and more hydrophilic hydroxylpropylene groups grafted onto the starch improved the compatibility between the gelatin and starch. SEM revealed a continuous phase of gelatin on the surface of films from all blends. The water contact angle of pure gelatin and the different blends were similar, indicating a continuous phase of gelatin. By optimizing temperature and incubation time to control viscosity, capsules of various blends were successfully developed. PEG increased the transparency and toughness of the various blends.
The complex issue of compatibility between starch and gelatin was investigated based on their interface and phase composition using synchrotron Fourier transform infrared (FTIR) micro-spectroscopy. A high amylose (80%) corn starch grafted with flexible and hydrophilic hydroxpropyl groups and plasticized by PEG was used throughout this work. The FTIR beam focused on a 5 μm × 5 μm detection region and the micro-spectroscopy was scanned across the gelatin–starch interface. It was found that there was about a 20 μm thick layer where gelatin and starch were in co-existence, indicating that gelatin and starch are compatible to a certain degree in these blends. The ratio of the areas of the saccharide C–O bands (1180–953 cm⁻¹) and the amide I and II bands (1750–1483 cm⁻¹) was used to monitor the relative distributions of the two components of the blends. FTIR 2 and 3-dimensional maps indicated that gelatin constituted the continuous phase to 80% of starch content. The PEG was homogeneously distributed in both gelatin and starch phases, and it blurred the interface between gelatin and starch in the chemical maps, indicating that PEG acted as a plasticizer and as a compatibilizer for the gelatin–starch blends.

Morphologies and phase compositions of different starch–gelatin blends were investigated by various microscopies: optical, SEM and synchrotron FTIR micro-spectroscopy. SEM revealed that the surface became smoother after adding PEG. Optical microscopy (OM) observation revealed that compatibility between gelatin and starch was improved by adding PEG. An FTIR beam focused on a 5 x 5 μm detection area by the micro-spectrometer was used to map chemical composition. The ratio of areas of the saccharide bands (1180–953 cm⁻¹) and the amide I and II bands (1750–1483 cm⁻¹) was used to monitor the relative distributions of the two components in the blends. All of the FTIR spectra showed contributions from both starch and gelatin absorptions, therefore indicating that complete phase separation into pure starch and gelatin domains did not occur. The PEG improved the compatibility of the gelatin–starch blends.
Because of the need to have a rapid test to identify gelatin and hydroxypropyl starch (HPS) in the blended films, a simple technique of identifying the HPS in the blend under an optical microscope (OM) through visualizing HPS with iodine was established. This method offered a direct and definitive way to study the approximate phase distribution of starch in the blends. By adopting this observation method and combining with SEM, FTIR and extensograph, the phase transition, miscibility and mechanical properties of the blends were studied systematically, and a relationship between phase transition, miscibility and film properties was established. Research using OM showed that phase inversion occurred when HPS ratio was 80% and interphase mixing was observed, which proved that these blends showed compatibility to some extent. FTIR and SEM further proved the compatibility of these blends. Contact angle showed a sharp change at an HPS ratio of 80% and modulus showed an inflection at this blending ratio, which were due to the phase inversion.

The influence of plasticizers on the multilevel structure, mechanical properties and transparency was studied. Plasticizer mechanisms acting on the mechanical properties were established. All the plasticizers increased the crystallinity, among which, glycerol had the most profound effect, followed by PEG, then propylene glycol (PG). The influence of plasticizers on the structure of suspended microcells depended on the type of plasticizer. PEG decreased the compactness of the self-similar structure, PG increased the compactness of the self-similar structure of all blends except for pure gelatin. Glycerol plasticized blends did not possess self-similar structure, but showed a lamellar structure with 15.7 nm spacing. The order of the extent of the influence of plasticizers on decreasing $T_g$ was PEG > glycerol > PG, indicating that PEG showed the greatest efficiency in increasing mobility within the amorphous region, followed by glycerol, then PG. PEG improved the mechanical properties of the blends to the greatest extent, followed by glycerol, then propylene glycol. The order of extent of the influence of plasticizers on increasing the transparency was glycerol > PEG > PG.
1. Introduction

1.1 Introduction

Gelatin exhibits good film-forming and gas barrier properties, and it has been used widely in the food and pharmaceutical industries. Gelatin capsules have been developed and used in pharmaceuticals since the early 19th century, and the technology has remained essentially unchanged [1, 2]. However, the well-recognized shortcomings of gelatin capsules, such as being an animal-derived ingredient, lower softening temperature and the instability of moisture content in gelatin, have led to attempts to use replacement substances, such as hydroxypropyl methylcellulose (HPMC) [3-6], poly(vinyl alcohol) (PVOH) [6,7] and modified starches [8-12]. Although there are many patents and publications on the development of various substances for capsules, few non-gelatin capsule products have entered the market. The reasons for the lower commercial success include the higher price of these new products due to requirements for new processing facilities, and/or more complex processing conditions. The current techniques and facilities used for manufacturing capsules were based on the gelatin. Therefore, the development of gelatin-based blends for capsule production using conventional technology, and overcoming the shortcomings of gelatin products has both scientific and commercial importance.

Starch is a common food ingredient and it has facile film-forming behaviour [13-17]. Both gelatin and starch have separately been widely used to develop edible films, and developing edible films by blending starch with gelatin has attracted much attention [18-27]. For example, films made of polysaccharides and proteins show better gas barrier (O₂ and CO₂) properties than any pure film. Previous research has shown that gelatin and starch are immiscible and that phase separation affects the rheological, processing and mechanical properties of their blends. In most cases, gelatin is present as a continuous phase even in starch-rich blends [18-21].
However, their compatibility can be improved by various methods. Other researchers [22] found that the time-dependent modulus of gelatin–starch gels was sensitive to the extent of gelatin crosslinking, as influenced by the thermal processing conditions. Under certain processing conditions, a gradual increase in starch content produced gels of lower elastic modulus and increased degree of microscopic phase separation. It is [23] reported that differential scanning calorimetry (DSC) thermograms of gelatin–starch films after an intense thermal blending showed a single glass transition temperature, indicating complete molecular miscibility of the components. Researchers [24] found that phase separation depended on pH since the charges on gelatin are pH dependant. It was reported [25,26] that higher processing temperature improving the permeability of gelatin–starch films. More recently, other researchers [27] reported that, at a certain concentration, sago starch and fish gelatin could form compatible films.

Starch is a polymeric carbohydrate consisting of anhydroglucose units linked together primarily through α-D-(1 → 4) glucosidic bonds. Although the detailed microstructures of starch are still being elucidated, it has generally been established [28-31] that starch is a heterogeneous material containing two microstructures: linear (amylose) and branched (amylopectin). Amylose is essentially a linear structure of α-1,4 linked glucose units, and amylopectin is a highly branched structure of short α-1,4 chains linked by α-1,6 bonds. The linear structure of amylose exhibits behaviour more closely resembling that of conventional synthetic polymers. Previous studies [29, 32-34] have found that amylose and protein could form an amylose–protein complex, which is stable to 90 °C in excess water solution. It is expected that such a complex will improve the compatibility in solution between gelatin and starch. Furthermore, hydroxypropylation has been widely used to improve the viscosity, transparency and stability of starch products. It is expected that the flexible and hydrophilic groups of hydroxypropylene groups are more compatible with gelatin. Another key property of
starch modification by hydroxypropylation is its toxicological safety and so it has been widely used as a food ingredient, as well as being used alone as capsule material.

Investigation of composition and interface of a blend using Fourier transform infrared (FTIR) micro-spectroscopy enables unique insight into the interface and morphologies of the blends since it is based on chemical contrast between constituents. For example, a FTIR micro-spectrometer was used to study the composition of gelatin–amylopectin blends prepared by extrusion [35]. FTIR two-dimensional maps were obtained based on the ratio of the peak areas of saccharide and amide bands. The role of the integrity of the starch granule in defining compositional fluctuations within the film microstructure that could control the performance of these blends was investigated. However, there is no report about the phase composition of films prepared from solution, that are expected to have more homogeneous structure. Furthermore, investigation on the interface of starch–gelatin blends, in particular polymer chain diffusion, could further explore the mechanisms of compatibility of the blends.

Mapping resolution depends on the size of the detecting region. Theoretically an FTIR beam concentrated in a smaller region will result in lower sensitivity for a typical FTIR spectrometer. Synchrotron FTIR has a much higher signal-to-noise (S/N) ratio and higher spatial resolution, which allows mapping of the microstructure, as well as providing insight into the chemical distribution and interactions [36]. Synchrotron FTIR micro-spectroscopy is a good technique to complement other techniques for investigating starch and/or gelatin phases in the blends, in particular chemical composition.

Gelatin will be blended with hydroxypropylated high amylose (80 %) corn starch to develop hard capsule materials. Poly(ethylene glycol) (PEG) was used as both a plasticizer and a compatibilizer in the blends. In order to prepare hard capsules for pharmaceutical applications by the well-established method of dipping mould pins into solution and then drying, solutions with higher solids concentrations (to 30 %) were investigated and developed. Films with
different ratios of gelatin–starch will be prepared by casting. The viscosity of various solutions will be studied by viscometry, and the mechanical properties of the films were studied by tensile testing. The morphologies and compatibility of gelatin and starch will be investigated by transparency, SEM and water contact angle. A synchrotron FTIR with micro-spectroscopy facility will be used to study the interface and phase composition of gelatin–starch blends of cast films. The contribution of PEG on the morphology of the blends is to be investigated based on the mapped composition.

1.2 Aim

The aim is to design and prepare various gelatin–starch blends used as edible packaging materials, in particular to investigate the morphologies, thermal, rheological, viscoelastic, phase separation and mechanical properties of gelatin–starch blends.

1.3 Objectives

Preparation of various gelatin–starch blends, then casting films.

Characterise suspensions and films using X-ray scattering, differential scanning calorimetry and optical and electron microscopy.

Compare the ratio of gelatin–starch on processability and suspension/film performance.

Determine and compare viscoelastic properties of the suspension/films.

Determine and compare mechanical film properties.

Study the microscopic characteristics and kinetic contributions to the state of phase separation in gelatin–starch blends.

Study influences of plasticizer on the phase separation of gelatin–starch blends. Compare any difference of the plasticizers affecting to the phase separation.
1.4 Research Questions

Will starch and gelatin be sufficiently compatible to be moulded into capsules and retain mechanical properties for capsules, even though they are known to be immiscible?

Can mutual plasticisers be found that will soften both starch and gelatin phases, and contribute to increasing compatibility?

Will there be an optimum ratio of starch and gelatin that provides enhanced properties or will there be a linear transition of properties with composition?

Gelatin has been found suitable for many commercial capsules, while starch based materials are used in packaging, so will the combination of starch and gelatin overcome the shortcomings of gelatin alone?

Since the polarity of starch and gelatin are different, a mixture of plasticisers may be better than a single plasticiser, or could a copolymeric plasticiser provide the best combination in a single material?

Will phase separation phenomena performance different in solutions with different concentrations?

Will plasticisers solve phase separation of gelatin–starch blends? How will plasticisers influence phase separation phenomena in solutions

1.5 Thesis Structure

This thesis contains 10 chapters. This chapter has provided an introduction to starch, gelatin and their blends, the aim, objectives and research questions to be addressed in the thesis. Chapter 2 contains a literature review of the materials, their properties and applications in forming materials. Chapter 3 presents the methods used in the research. Chapter 4 details compatibility and phase transitions of gel–HPS blends. Chapter 5 investigates starch–gelatin blends phase composition and interface. Chapter 6 examines phase inversion and compatibility.
of HPS–gelatin blends. Chapter 7 features the morphologies and phase composition of HPS-gelatin blends. Chapter 8 explores the action of plasticizers on microstructure and mechanical properties of gelatin–HPS blends. Chapter 9 details final results and conclusions and a brief discussion of where research could be extended.

![Figure 1-1: Structure of thesis](Image)
2. Literature Review

2.1 Progress in edible packaging materials

2.1.1. Applications of edible membranes

Consumers demand high-quality food that is safe and consistent. To satisfy consumer demand, researchers are committed to exploring new methods of maintaining food quality, freshness, and safety, such as the use of renewable natural materials for edible membranes and inner-lining of food packaging materials [1]. Investigation of edible membranes has grown over the last 20 years. Researchers have widely studied the practicability of biopolymer-forming membranes, thus developing solid fundamentals for the preparation of edible membrane food packaging [2]. Edible membranes must offer good elasticity and ductility, as well as low fragility and high strength. They must not fracture or fail during transportation and storage [3]. Biopolymers such as polysaccharides, proteins and lipids have been used as raw materials for edible membranes. There have been many studies on the use of lipids [4], proteins [5], and polysaccharides (such as chitosan [6], hemicellulose [7], and starch [8]) as edible membranes, as well as on the development and application of edible membranes themselves [9-14]. Edible membranes based on gradients can be categorized as hydrophilic colloids (proteins and polysaccharides), lipids (fatty acids, acylglycerols, and waxes), or composite membranes.

Edible membranes were first developed in the 1960s, primarily to extend the shelf lives of meat products. Later, they were widely applied to improving the quality of fish, frozen food, fruits and vegetables. Some recent articles have commented on the application of edible membranes to the preservation of fruits and vegetables. Studies show that they can reduce mechanical damage [15, 16] to fresh produce during transportation. For example, frozen food and fresh fruits and vegetables wrapped in edible membranes experience stronger support. Thus, mechanical damage is reduced during handling and treatment.
Edible membranes serve to block water vapor and retain subtle flavors. Gasses and vapors pass through homogeneous membrane materials via active diffusion (the gas within the membrane diffuses based on solubility and concentration differences, and is transported to its exterior). Therefore, penetrability is determined by a combination of membrane material permeability and environmental factors such as concentration, temperature and humidity [17, 18]. In a food system with multiple components, water vapor diffusion is the most significant source of mass transport between constituents, and it may cause desirable sensory characteristics to be lost [17]. Thus, edible membranes should limit moisture migration. This is vitally important for maintaining the quality of multi-phase foods [17, 19].

Hydrophilic, high molecular weight polymers, and polymer electrolytes (alginate, carrageenan, carboxymethyl cellulose, pectin and xanthan gum) are widely used in membrane formation materials to control and protect the texture, taste, and shelf life of food [22]. Since these polymers are water-soluble, they offer less obvious moisture-barrier capabilities, particularly in humid environments. However, they can block and protect lipids, as well as prevent lipid oxidation.

Some edible membrane materials, particularly those comprising hydrophilic macromolecules, are effective barriers to fats and vegetable oils. Such membranes can reduce the oil absorption rate when used as coatings for fried foods, improve nutritional function, and reduce fat and calories [20]. Since an increasing proportion of the population suffers from obesity and coronary heart disease, it has become increasingly important to use edible membranes that can reduce the fat content of food. The purpose of frying is to seal the food surface in order to retain flavor and moisture. Frying involves the transmission of thermal energy and quality (fat migrates into the food while moisture migrates out). In deep-frying, moisture evaporates from the surface and other moisture migrates from inside the food to the surface and then evaporates. The voids formed by moisture evaporation provide grease with access to the interior of the food.
Loss of moisture is closely related to fat absorption. The micro-structure of the surface is key to determining fat absorption. The absorption occurs via a capillary mechanism. The edible membrane toughens the surface texture of the food, restricting formation of large voids, and hence reduces moisture evaporation and fat absorption [21, 22].

Edible membranes can be used as carriers for some food additives. There have been many studies on the addition of antiseptics to edible membrane materials. Antiseptics can prevent or reduce the growth of microorganisms on the food surface, extend shelf-life, and enhance food safety. Other additives such as antioxidants, anti-browning agents, nutritional health products, reinforcing agents, flavoring agents, and pigments can be added to an edible membrane to enhance its ability to protect the food while intensifying its functionality and sensory characteristics [13-17].

2.1.2. Progress in gelatin-based edible film research

Gelatin is a partial hydrolysate of collagen formed in acidic, alkali, or enzymatic environments, or in high temperatures. Gelatin has both acidic and basic features, and is amphoteric. It undergoes micellar electrophoresis and migrates towards positive or negative electrodes under the influence of an electric field. Its isoelectric point is pH 7–9 after acid hydrolysis and pH 4.6–5.2 after alkali hydrolysis. Gelatin is composed of 18 different amino acids, but it is not a homogeneous protein as its molecular weight ranges from 50,000–70,000 g/mol. Gelatin from yellow colloids is extracted from the connective tissues of animals. It is not easily dissolved in cold water, but can slowly absorb 5 to 10 times its mass of cold water, forming a strong, elastic gel. Gelatin is easily dissolved in warm water. Since it absorbs water well, it can be used as a gelled support frame. When gelatin is dissolved in warm water, it gradually swells and then attracts other gelatin molecules, interweaving with them to form three spiral mesh structures. As the temperature falls, it condenses to form an elastic gel.
Gelatin molecules contain many hydroxyl, carboxyl, and amino groups. This makes them strongly hydrophilic and reactive. Since gelatin is bio-degradable, bio-compatible, and histologically compatible, it can be used as a non-toxic substance invitro after degradation. Gelatin can be used in biopolymer and biomedical materials, and is widely applied within food technology, pharmaceuticals, and other fields[22].

A common method of preparing edible gelatin films is to mix gelatin with water, heat it to its swelling temperature until it forms a uniform paste, stir it until it is completely dissolved, and add plasticizers, crosslinking agents, and other substances as required. The resulting product is then defoamed, allowed to stand, and stirred well before being daubed or dumped into a container. The film is then dried and stripped from the container surface[33]. Food can be placed in the solution and removed after drying to produce film-coated food. Edible gelatin film quality is affected by several factors, such as solution concentration, pH, plasticizer, crosslinking agent, metal ions, and enzymes[35]. Gelatin film quality is usually evaluated via its modulus, tensile strength, elongation at break, barrier properties, water vapor transmittance, solubility, swelling, and water retention[37].

Gelatin is fibrous and contains a triple helical structure. A network structure is formed by crosslinking the chains with water to fill intermolecular gaps. When gelatin gelatinizes, water is squeezed from the protein matrix and it shrinks to form a rubbery film. It then transforms into a glassy gelatin film after drying. Additives such as plasticizers and crosslinking agents are used to improve its gel properties in order to produce better performing, edible gelatin films. Plasticizers typically reduce the brittleness of gelatin films, but decrease their mechanical strengths and thermal stabilities. Crosslinking improves the mechanical properties and thermal stabilities of the gelatin films, and slows their degradation rates. Plasticizer selection usually requires considering gelatin compatibility, film permeability, and the amount of plasticizer added [7]. Common plasticizers include glycerol, mannose, sorbitol, poly(ethylene glycol), and
ethyleneglycol. Edible gelatin films are typically crosslinked using enzymes, chemical crosslinking agents such as glutaraldehyde, or physical crosslinking methods[11].

Hydrophilic plasticizers form hydrogen bonds with protein chains, reducing intermolecular bonding between proteins [8] and thus the elastic modulus \((E)\) and tensile strength \((TS)\) of the gelatin film, but increasing its elongation at break \((EB)\). Plasticizers usually impose two plasticizing effects: the first is that of the plasticizer itself, and the second is that plasticizers are significantly hygroscopic and can absorb water into the gelatin matrix structure [9, 10]. Arvanitoyannis et al. [11, 12] compared the recombination of gelatin with soluble and hydroxypropyl starches. When the edible film was plasticized with a polyol, it exhibited an increase in water absorption. Glycerol and sorbitol reduced the \(TS\) and \(E\) values of the films, while increasing their \(EB\) values. According to Sobral et al. [13], when sorbitol is used to plasticize a gelatin film, its puncture strength declines as the sorbitol concentration increases. Accordingly, puncture deformation and the water vapor permeability coefficient increase, the glass transition temperature range broadens, and phase separation phenomenon appear. Lim, et al. studied [14] glycerol performance on glutaminase-crosslinked gelatin films. As the amount of glycerol increased, the film water content, \(EB\), and oxygen transit dose increased, but \(TS\) was reduced. The research of Lin Haili et al. [37] showed that the \(TS\) of a gelatin film fell, but \(EB\) and flexibility increased when the amount of glycerol increased. Ethanol addition affected the gelatin film drying speed and intermolecular network structure. Increasing the ethanol dosage to 10% (relative to gelatin) increased the \(TS\) of edible gelatin films, while adding more ethanol decreased the strength. Vanin et al. [45] compared the influences of glycerol, propylene glycol, diethylene glycol, and ethylene glycol on the mechanical strengths of gelatin films. Glycerol had the greatest influence on the resulting mechanical properties. Thomazine et al. [15] studied the mechanical properties and water resistance of gelatin films with different ratios of glycerol and sorbitol as plasticizers. Plasticization with a mixture of glycerol and sorbitol resulted in
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glycerol elimination and plasticization with sorbitol. The plasticizer can be easily precipitated from the film.

Plasticizer addition usually reduces the thermal stability of a gelatin film. Barreto et al. [16] found that plasticizers such as sorbitol significantly reduce the degradation activation energy of sodium casein, whey protein, and gelatin edible films by reducing the initial and highest temperatures of thermal degradation. The decline in thermal stability is related to the influence of sorbitol on intramolecular and intermolecular protein hydrogen bonding. Goswami et al. [17] found that recombination of gelatin and trimethyl phenol causes the thermal stability to increase. When poly(ethylene glycol) (PEG) 400 was used as a plasticizer at less than 7.7 %·w/w, the thermal stabilities of the resulting materials continued to increase, but fell when this dosage was exceeded. This occurred because PEG 400 can contain hydroxyl or methoxy end groups, as well as many chain-linking ether groups, and its impact on thermal stability is complex.

Water is an effective plasticizer for gelatin films. In addition, moisture absorbed in the films affects the plasticizing capabilities of other molecules. The molecular structure and composition of each plasticizer affects its ability to destroy hydrogen bonds between protein chains and absorb water into the protein system. Yakimets et al. [18] investigated the influence of different water concentrations on the mechanical properties of glassy gelatin films. They proposed that hydration be divided into three sections: (1) water bound in the high-energy absorption area, (2) constituent water, and (3) water between multi-molecular layers. Gelatin films are brittle below their glass transition temperatures. When the moisture content was 7–14 %, the degree of gelatin renaturation into collagen was high, so the mechanical properties of the gelatin films increased. Lukasik et al. [19] used a phosphor to monitor the molecular motion of water in polyol-plasticized gelatin films. They discussed the influences of the plasticizer and physical crosslinking on molecular motion and oxygen permeability in the gelatin films.
The hydrophilic nature of gelatin films renders them susceptible to water penetration, which reduces their mechanical performance and barrier properties. Gelatin does not offer good packaging performance unless enhanced by chemical agents or strengthened with other composite-forming materials. The performance of a gelatin film can be improved by producing a composite with starch, cellulose and lipids. When gelatin is modified via blending or copolymerizing with another polymer, its performance can be increased to meet the specifications of various products, and used where its natural advantages are most apparent.

Compatibility between components of a composition is significant in compounding. Component compatibility can be evaluated by measuring the transition temperature ($T_g$) via differential scanning calorimetry (DSC). Confirmation of compatibility between matrix polymers is confirmed if a binary mixture exhibits a single $T_g$. If there are two $T_g$ values with positions different from those of the matrix polymers, a heterogeneous blend has been formed. Jagannath et al. [42] combined gelatin and starch using both hot and non-hot-mixing methods to form edible composite films. They found that there were two $T_g$ values after non-hot-mixing, but only one after hot-mixing, thus demonstrating compatibility between the components. Arvanitoyannis et al. [11] reported that for composite films containing water, glycerol, sorbitol, gelatin, and starch plasticized with sucrose, the $E$ and $TS$ values decreased, but $EB$ values and gas permeabilities increased when the plasticizer concentration was increased. A film dried at a low temperature (20 °C) had a higher crystallinity and lower gas permeability than one dried at a higher temperature (60 °C).

Li et al. [43] prepared composites consisting of konjac, glucan-mannan, and gelatin to form a rapidly dissolved edible film that could be heat sealed. Xiao et al. [44] found that the thermal stabilities and mechanical properties of composite films increased, and that brittleness was improved. Lee et al. [45] combined gellan gum and gelatin into films with excellent mechanical properties. Dong et al. [46] alloyed alginate and gelatin by crosslinking them with a calcium salt,
and applied the product to a controlled drug release system. The $TS$ and $EB$ values of the composite films reached their maxima when the gelatin content was 50 %·w/w.

Two advantages of edible gelatin films are their good biocompatibility and biodegradability. As a natural polymer, gelatin has no antigenicity, and can be fully absorbed by the body. Several functional groups can be used to chemically crosslink gelatin, and its physical and chemical properties can be adjusted. However, it has shortcomings such as poor mechanical properties and poor barrier performance in specific applications. Gelatin film performance can be improved by adding a plasticizer, crosslinking agent, or other natural or synthetic polymer.

2.1.3. **Progress in starch-based edible films**

Starch can be divided into amylose and amylopectin. The first is a linear polymer with repeat units of glucose linked by $\alpha$-1,4-glycosidic linkages, while the second has an $\alpha$-1,6-glycoside branch.

Starch films offer advantages such as high transparency and absence of color and taste. They have water solubility and low permeability [48-49, 50, 51], but exhibit strong hydrophilicity and inconsistent mechanical properties [52], along with rapid physical aging and revival. The water resistances of starch-based membrane materials can be improved by adding lipids or other polymers, and their mechanical properties can be enhanced via mixing with clay [52, 53], fibers [52, 54], particles [55], or nanoparticles [53-56]. The ease with which the films are physically aged and revived leads to crystalline and amorphous regions. Thus, the physical and chemical properties of starch films, such as their tensile and gas barrier properties, are affected by the cohesion energy densities of the two regions. The crystalline of native starch increases with its amylase pectin content, while starch films have low crystalline. The crystalline of starch increases with its amylase content [57]. Dextrinization produces recrystallization, and some molecular chains transition from amorphous to crystalline states [58]. During aging, amylose molecules easily form ordered structures, helical structures, and crystalline textures.
When starch is dissolved in hot water, amylose and amylopectin lose their crystalline structures to form hydrates. Later, macromolecules rearrange and amylose and amylopectin form crystals via hydrogen bonding during membrane formation. The resulting crystallization of starch-based membrane materials is connected to several factors such as drying, storage (temperature and relative humidity), and plasticizer [60].

Blending is a good way of improving the physical and chemical properties of starch films and decreasing film aging. It can also enhance the mechanical and hydrophilic properties of the films. For example, starch can be blended with agar, chitosan, and cellulose. Wu et al. [61] and Phan et al. [62] studied the properties of packaging materials made from agar and starch. They used infrared spectroscopy to prove the intercompatibility of the two materials. Agar improved the microstructures of starch films, and increased their tensile strengths and moisture barrier properties in high-humidity situations. Ghanbarzadeh et al. [63] proved that carboxymethyl-cellulose-starch-glycerol blended membranes have better mechanical properties than pure starch membranes. However, Müller et al. [64] found that fiber-containing membrane materials exhibited increased crystallinities, strengths, and hardnesses. They also found that fibers increased the stabilities of the starch-based membranes at a range of humidities. Other studies have considered the influence of chitosan on starch-based membrane properties [63-69] and the mold resistance of starch–chitosan blends. After adding chitosan, the solubility, oxygen and water vapor barrier properties of starch-based membranes decreased, although their mechanical properties increased. Fernandez et al. [70] studied the performance of chitosan–starch blends and found that membrane materials made from blends with Hylon VII and chitosan were stronger and tougher than those made from corn starch containing 28% amylose because Hylon VII contains 70% amylose. Also, Jimenez et al. [8] proved that the addition of hydroxypropyl methylcellulose significantly reduced the physical aging and moisture-vapor transmission of starch.
2.2 Structures and functionalities of edible membrane materials

The study of packaging layer materials can be divided into two categories: analysis of the structure of the packaging membrane material and testing its functionality. Structural analysis uses various microscopic and spectral techniques, while functional tests vary by the application [71].

2.2.1. Design and structure

2.2.1.1. Material morphology

Microscopic techniques such as scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), wide-angle X-ray scattering (WAXS), and small-angle X-ray scattering (SAXS) are used to assess the morphologic functions of membrane materials. The microstructures of edible membranes were studied via SEM and CLSM. The mechanical and barrier functions of biopolymers are related to their microstructure, which depends on structural gradients and the process used to prepare the membrane [72]. Ogale et al. [73] proved via SEM imaging that the longitudinal section of a soybean protein layer is quite rugged, which suggests substantial membrane plasticity [73]. McHugh and Krochta (1994) [74] used SEM to assess the longitudinal structure of a wheat protein plasticized with sorbitol. Their study showed that eliminating the bubbles in solutions via application of a vacuum reduced the size of voids in the membranes, thus reducing the water vapor transmission rate [75]. Various studies show that the presence of structural defects such as pinholes and cracks affects the barrier function of membranes. Giancone et al. [77] used SEM to investigate the effect of surface density on the surface morphologies of pectin-based edible layers with high methoxyl content. Their study showed that the formation of pectin clusters lead to an inhomogeneous pectin membrane structure. However, surface density had no effect on the microstructure of the membrane. Jin et al. [78] used CLSM to study the surface structures of pectin and PLA membranes to which
nisin had been added. Such technology helped to produce high-definition optical images and achieve layered scanning and rebuilding of the three-dimensional structures of irregular and complex-shaped objects. The CLSM observations of Jin et al. [78] showed that pectin–PLA membrane materials exhibit relatively tough structures, and that the activity of nisin in composite membranes is relevant to the toughness of the membrane materials. The toughness and hydrophilic nature of pectin aid in the penetration and adsorption of nisin.

WAXS and SAXS were used to study the crystalline and aggregate structures of membrane materials. Gohil et al. [79] studied the crystal structures of membranes made from blends of pectin and sodium alginate with a small amount of methoxyl after processing with calcium chloride. Sodium alginate exhibited an irregular structure, while pectin exhibited low crystallinity. Thus, the crystallinity of the blended membrane increased with the pectin content.

### 2.2.1.2. Interfacial interactions and membrane material compatibility

Composite membrane material functionality depends primarily on the appearance, size and distribution of composition gradients [80]. Factors influencing the appearance of the blended materials include composition, interfacial tension, blending conditions, and the rheological functions of the composition gradients. Polymer blends can be divided into scattered, layered and continuous morphological structures by appearance. Liquids decrease spreading in continuous media. The interfacial morphologies of the membranes are important to the compatibility and miscibility of the materials.

Miscibility depends on whether the free energy of mixing is below 0, i.e. it is required that \( \Delta G = \Delta H - T\Delta S < 0 \). The entropy change during blending of high molecular weight polymers is small, and the mixing process absorbs heat, i.e. \( \Delta H \) is positive. Hence, it is difficult to meet the requirement that \( \Delta G < 0 \). Since \( \Delta G \) is often positive, most blended polymers are not miscible at the molecular level thus form heterogeneous blends, even though they may be compatible.
There are many ways of determining material compatibility such as microscopic structural observation, glass-transition temperature, cloud point temperature, infrared spectroscopy, and light scattering. The most intuitive of these is microscopic observation.

Carmen-Alice Teac [81] et al used fibers to mechanically reinforce modified starch systems. Their study showed that there is some compatibility between starch and the reinforcing fibers, as demonstrated by the improvement in mechanical properties. The study conducted by Jimenez et al [82] showed that phase separation occurred in corn-starch–HPMC blends and that homogenizer treatment aggravated the extent of separation. Moren et al [83] studied compatibility within starch and buttermilk blends. The blends exhibited low compatibility, as reflected by their phase-separated morphologies. The study conducted by Xi et al [84] showed that starch–cellulose acetate blends embedded with epoxidized soybean oil and plasticized with glycerol exhibited compatibility. Liu with partners [85] and Zhang with partners [86] used optical microscopy, SEM and synchrotron Fourier transform infrared (FTIR) microspectroscopy to study phase separation and morphology in starch–gelatin blends. The results indicated that starch–gelatin blends are completely incompatible, but that the phase inhomogeneity observed via SEM improved significantly after PEG was added. This showed that addition of PEG improved the compatibility of the system. In addition, results of experiments conducted with synchrotron infrared radiation showed that PEG is miscible in both the starch and gelatin phases, thus enhancing the compatibility between the two phases.

2.2.2. Membrane material functionalities

2.2.2.1. Rheological properties

Gelatinization occurs when starch is heated in a dilute aqueous solution [87]. When the temperature exceeds the gelatinization temperature, the starch particles swell, hydrogen bonds between starch chains in the amorphous region break, and the starch chains become hydrated.
As the temperature increases, starch chains in the crystalline region undergo gradual hydration and the crystal structure finally melts. The melting of crystals, double helices, layered structures and particles are all irreversible [88].

Many studies have shown that starch paste gelatinization is dependent on starch type [89-94]. Sasaki et al [94] and Techawipharat et al [95] found that the amylose content controlled starch and starch paste gelatinization. The nature of the gelatinized starch depends on the ratio of linear- to side-chain starches [95], the molecular weight distributions of the two starches, the quantities of residual proteins and lipids in the starches, concentration, temperature and stirring during heating and cooling.

Starch paste is a type of thixotropic, shearing-diluting fluid [96]. Starch–gelatin dispersions are often meta-stable, with a phase size dispersion that arises from the intensity of shear mixing [97]. Over time, agglomeration can occur and produce a larger phase size dispersion, and the starch can produce some crystals [98]. In hot starch paste, amylose molecular chains can intertwine to form a network structure and exhibit obvious solid-like behavior, becoming elastomeric and gelatinized during cooling, especially when a high amylose content is present [99-103]. Such a process can last for 48 h. The amylose ages and precipitates at low concentrations. The residual starch particles or debris after gelatinization serve as a filling phase in the starch–gelatin network [104-107]. The intertwining of some side-chain starch is conducive to the formation of gelatin films. However, since the side-chain starch aging process is very slow and is tied to storage temperature, it can take several weeks for gelatinization to complete [103-107]. Molecular chains made from side-chain starches can combine to form relatively weakly linked gelatin in systems that contain no linear-chain starch [108-109]. The longer side chains in the side-chain starch can reduce the time required for gelatin formation [110]. Various hydrogels have different effects on the starch system, since they exhibit different structures (chemical structure, ionic charge, shape, rigidity/flexibility, molecular weight, and
degree of branching) and characteristics. In addition, interactions between the gelatin molecular chains and water molecules can have an effect on the starch system [111].

The transformation between gelatin and suspension can be tested using a force- or change-responding rheometer that can show the storage and loss moduli. The storage and loss moduli exhibit different trends at different stages in the gelatin formation process. They increase slowly during the initial phase, rapidly in the middle phase, and slowly again in the final phase. Frequency scanning can be used to study the solid-like and elastic behaviors of gelatin, while temperature scanning can be used to study the depolymerization process. Frequency and change response tests can be used to judge the strength of gelatin [128]. Both small-angle neutron scattering and SAXS can be used to study the structure of gelatin. Ross-Murphy et al [129] used SAXS to study the gelatin structure of carrageenan. The study shows that the carrageenan solution contains worm-shaped polyelectrolyte chain segments [130], yet the gelatin network contains a double helix-structured, bundle-shaped material. The average diameter of the linear polysaccharide aggregates in the gelatin network structure could be calculated via SAXS and neutron scattering, as could information about the homogeneity and hole size of the gelatin structure. Some researchers [131] have used X-ray and neutron scattering to study the molecular aggregates and gelatin structure of lactoglobulin at various concentrations, pH, and ionic strengths. X-ray scattering can also be used to study the fractal and network structures of gelatin aggregates, as well as the homogeneity of gelatin.

Typically, the blending system becomes stratified and phase separation occurs, resulting in formation of large, heterogeneous structures before and during gelatin formation. This synergistic reaction takes place in polymer blend systems. Morris et al [132] conclude that synergistic reactions cannot be precisely defined, nor can any specific method be established to study them. However, the results from a blending system that exhibits synergistic reactions must exceed the sum of the contributions from each individual gelatin to the system. In this
study, phase-separated gelatin may produce synergistic reactions due to higher local concentrations caused by phase separation. However, Morris et al. [132] explain that this is not the common definition of synergistic gelatin. Synergistic reactions are phenomena in which two types of polymers combine with each other at the molecular level to form a new, coupled network structure instead of gelatin. Few applied systems exhibit such phenomena. However, a study of a blend of locust bean and xanthan gums conducted with a rheometer, microscope, and DSC showed that a synergistic reaction occurred [133]. In contrast, no such synergistic reaction occurs in the locust bean gum–K-type carrageenan blending system [134].

It is common for phase separation phenomena to occur in a blending system that contains two types of polymers. Such phenomena include each of the polymers forming its own phase and the appearance of condensed heterogeneous phases in each polymer. Polymer intercompatibility is closely associated with the interactions between the polymers and the solution. If the affinity between one type of polymer and the solution differs greatly from that between the other type of polymer and the solution, the two kinds of polymers will be incompatible. Thermokinetic incompatibility and phase separation are mechanisms for changes in the composition of gelatin. Phase separation can be observed in gelatin–t-type carrageenan blends, and is caused by condensation [135]. Dave et al. [136] studied hyaluronic acid–hydroxypropyl cellulose blends, in which more severe phase separation occurs and leads to the formation of a heterogeneous phase. In recent studies, researchers used FTIR microscopy and laser confocal microscopy to study phase separation, as well as the gradients and volumes of each phase [136]. However, insufficient resolution has limited these studies [137]. Tromp et al. [138] used a position-sensitive small-angle light scattering technique to track and study the dynamics involved in gelatinization of glucan–gelatin blends. The blends were analyzed based on rotating node and scaling theories. The results showed that the kinetic parameters became quite different in the vicinity of the gelatin formation critical point. Condensation leads to phase
separation in the gelatin–κ-carrageenan blended system. Brown et al [139] studied the effect of shearing on phase separation and the gelatin structure. A study conducted by Kalichevsky et al [140] found that linear-chain starch and glucan remain separate in their blending process. κ-type carrageenan–corn side-chain starch blends exhibit extensive phase separation. Yet, phase separation occurred only during gelatinization in the κ-type carrageenan–corn linear-chain starch blending system[141, 142]. However, Sikora et al [143] and Funami et al [144] did not find phase separation in the carrageenan–starch blended system. The study conducted by Lai et al [145] showed that adding starch promoted the formation of κ-type carrageenan gelatin. Mohammed et al. [146] blended waxy corn starch with agarose, and found that swollen particles appeared as a filling phase in the homogeneous gelatin network when the starch concentration was 2%. Double continuous phases were formed when the starch concentration was 3–5%, and the starch formed a continuous phase when its concentration reached 6%. There are strong interactions between starch and Hsin-tsao leaf gelatin [148-155]. The study conducted by Chen et al [149] showed that a composite structure was formed between starch and Hsin-tsao leaf gelatin. The study carried out by Michniewicz et al [156] showed that the starch polymer (side-chain starch) and water-soluble pentosan can interact with each other to form a complex substance.

2.2.2.2. Study of membrane material functionality

The study of material functionality is at least as important as that of membrane structure. There are ever more studies related to the functionalities of packaging membrane materials, such as mechanical and barrier functionalities, and how they fit the main goals of food packaging. Chambi and Grosso [162] proposed simulating the structures of organisms. One recommendation is to use the cell walls from plant tissue as a source of degradable membrane material with improved mechanical properties. For instance, methyl cellulose, glucomannan, and pectin can be blended to produce a degradable membrane. After using ASTM D882 to
study the mechanical functionalities of membrane materials, it was concluded that the membrane material with the best mechanical functionality was a 1:4:1 methyl–glucomannan–pectin blend (tensile strength 72.63 MPa and elongation at break 9.85 %) [162]. Some researchers have studied the effect of nanofibers on the mechanical functionalities of edible membranes prepared by blending pectin with mango puree [163]. The study showed that adding nanofibers can improve mechanical functionality. The improvements in mechanical functionalities became clearer when more nanofibers were added. Some researchers have performed studies on the effects of antiseptics on the mechanical functionalities of edible membranes [164]. Du et al. [164] studied the effects of sweet pepper, cinnamon and essential fatty acids from Syzygium aromaticum, on the mechanical functionality of an edible membrane made from a blend of apple butter and pectin. Adding the essential fatty acid reduced the mechanical functionalities (mechanical strengths and moduli) of the films ($p< 0.05$). The essential fatty acid from Syzygium aromaticum had the most obvious effect on the mechanical functionality of the membrane material.

The functionality of the edible membrane changes over time. Mali et al [165] and Jiménez et al [166] all found that moisture transmittance through starch membranes changes significantly with time. A study conducted by Liu et al [167] showed that increasing the crystallinity of starch caused a reduction in the carbon dioxide transmittance of a membrane after storage. Another study conducted by García et al [168] showed that a membrane material made from corn and high linear-chain corn starch exhibits a lower carbon dioxide transmittance after storage (20 d at 20 °C and 63.8 % relative humidity (RH)) than when it was newly prepared. Carbon dioxide penetrated faster than oxygen, primarily because it was more readily soluble in the water within the starch membrane. Gontard with partners [169] and Alves with partners [170] found similar phenomena with wheat-based edible protein membranes and the carrageenan–pectin blending system.
2.3 Processing conditions on structure and functionality of edible membranes

2.3.1. Effect of solution preparation on the structures and functionalities of edible membranes

Some solution preparation factors such as blending method, stirring speed, rate of temperature change, and temperature and duration of placement all affect the nature of the edible membrane prepared. This is especially true when an edible membrane is prepared from a high-concentration solution. This is because solid-like gelatin (which is thixotropic and can flow in the high-vibration environment used in membrane preparation) is formed in some high-concentration polymer solutions at certain temperatures. The gelatin formation process can easily be affected by these processing conditions.

Cooling rate contributes to the rheological functionality of the membranes, resulting in membranes with different structures and abilities. A study conducted by Rao and Cooley [171] showed that the pectin–gelatin structure was controlled via the cooling conditions used. Slow cooling can contribute to the formation of a more complete and compact gelatin structure [172]. Preparation methods can affect the gelatin functionalities of the blending system. A study conducted by Closs et al. [173] showed that solution blending caused phase separation, while a system formed via powder blending and then dissolved exhibited no obvious phase separation. Wax-based corn starch–cold gel blend systems exhibited different functionalities after blending at different temperatures [174-176], since the network structure of cold glue scattered more homogeneously at 90 °C.
2.3.2. Effect of drying conditions on the structures and functionalities of edible membranes

Drying is one of the most important membrane preparation steps [178, 179]. The solution concentration, drying equipment, temperature, humidity, time and other factors, all affect the functionalities of the final membrane. Some researchers have dried thin membranes at room temperature, while others have dried membrane materials using ovens, microwaves, infrared energy, vacuum conditions and low-pressure heating [177, 178, 180, 181]. Several studies have considered the effects of drying conditions on alginate, gelatin, lactalbumin, chitosan, soybean proteins, and linear- and side-chain starches [182-192]. The optimal drying conditions depend on the characteristics of the raw material, such as the gelatin phase state before drying and whether formation or depolymerization of gelatin occurs during the drying process. In addition, other phenomena may accompany drying, such as conversion from a rubbery state to a glassy state, phase separation or recrystallization. The effects of drying conditions on the functionalities of polymer membrane materials are closely related to the physical and chemical properties of the polymers [184]. Menegalli et al [193] found that drying at higher humidities and temperatures can cause gelatin to melt, which eventually leads to a decline in drying efficiency, as well as phase separation. With methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC), high-temperature drying can cause gelatin formation, thus producing membrane materials with different characteristics. A study of starch membranes showed that drying conditions have particular effects on the crystallization and mechanical functionalities of membrane materials. A study of protein membranes showed that drying conditions have important effects on the functionalities of the final material, primarily because the proteins can easily be affected by processing parameters or become denatured [184]. The moisture transmittance of a lactalbumin membrane was reduced, and the solubility of the membrane material was enhanced by lowering the drying temperature from 25 °C to 5 °C. Alcantara et
al[182] verified that fast drying can enhance the mechanical and barrier functionalities of lactalbumin membranes. With chitosan membranes, the drying temperature has a more significant effect on mechanical and barrier properties than the humidity [184]. Compared to low-temperature drying, drying at 50 °C shortened the drying times of chestnut starch–carrageenan blends and improved the mechanical functionality of the blended membrane material [194]. Thus, drying conditions can have important effects on the final functionalities of the membrane materials.

2.4 Effect of plasticization on the structures and functionalities of edible membranes

Generally, plasticizer is added to a membrane material to improve its ability to function physically. The plasticizer can reduce the fragility and enhance the ductility of the membrane material, as it can decrease the interactive forces between molecules and increase the mobilities of polymer chains [193-196]. The addition of a plasticizer can have significant effects on the barrier ability of the membrane material. In most cases, a plasticizer is needed to prepare edible membranes, especially when polysaccharide- or protein-based membrane materials are used, as their molecular chains can easily undergo molecular interactions that cause the resulting membrane to be very fragile [198].

The most widely available plasticizers for edible membranes include polyols (such as glycerol, sorbitol and PEG), monosaccharides (such as glucose and fructose), and disaccharide (sucrose). Glycerol, PEG, and sucrose are the most commonly used substances. They can interact with the membrane material and reduce the risk that it converts to a glassy state [199]. Plasticizers often absorb moisture. Water can be used as a plasticizer, but it is unstable in air with low humidity and can vaporize easily [200]. In addition to improving the mechanical functionalities of the materials, plasticizers can affect the gas-barrier properties of the membrane [196]. Hydrophilic
plasticizers can enhance the transport of water vapor and aromatic gases [198]. The plasticizer quantity and type have substantial effects on the physical functionalities of membrane materials. Generally, a plasticizer with small molecular weight can enter the membrane material more easily than one with large molecular weight, thus exhibiting better plasticization [196]. The main functions of a plasticizer are to soften the membrane material, reduce its tensile strength, and enhance its elongation at break. An effective plasticizer should be highly compatible with the substrate. In such circumstances, glycerol is the most commonly used plasticizer for membrane materials. The plasticizer content and type, source of plant starch, and storage conditions can cause degradable polymer membrane materials to exhibit different mechanical functionalities. Generally, starch–glycerol blends exhibit low tensile strengths and moduli. Thus, the membrane material made with glycerol is softer than one made with sorbitol, and exhibits stronger ductility, which indicates that glycerol is a more efficient plasticizer. A similar effect is present in other systems, such as a blend of protein and sodium caseinate [200]. This is because glycerol is more hydrophilic than sorbitol, and the moisture absorbed can be used as additional plasticizer. In addition, glycerol can quickly diffuse into the polymer chains and interact via hydrogen bonding since it is a small molecule. It can also reduce molecular interactions and enlarge the gap between molecules, thus lowering the tensile strength and enhancing elongation at break. A study conducted by Dias et al. [201] showed that, regardless of whether glycerol or sorbitol was used as the plasticizer, moisture transmittance increased with plasticizer content. The starch membrane plasticized with sorbitol was better at barrier water vapor than its glycerol-plasticized counterpart. This is because glycerol has a stronger hydration function, while sorbitol was relatively weak in moisture absorption. Starch–sorbitol membranes had better gas barrier functionalities than starch–glycerol membranes [202]. García et al [203] studied the nature of MC edible membranes with sorbitol as the plasticizer. The membrane material made without plasticizer had several cracks, while the addition of a plasticizer produced a membrane material with better integrity. Sothornvitd et al [196] assessed
the effects of such plasticizers as propylene glycol, glycerol, sorbitol, poly(ethylene glycol) 200, poly(ethylene glycol) 400, and sucrose, which have molecular weights of 76, 92, 182, 200, 342, and 400 g/mol, respectively. These plasticizers rank as follows (from high to low) in terms of their efficiency in enhancing the elongation at break and lowering the tensile strength of lactoglobulin membrane materials: glycerol, sorbitol, poly(ethylene glycol) 200, sucrose, poly(ethylene glycol) 400, and propylene glycol [196]. Similar trends are observed with caseinate [205] and fish-skin proteins [206]. The polarity of the plasticizer can affect the functionalities of the membrane materials. A low-polarity plasticizer cannot provide good sites for hydrogen bonding with polymer chains, and thus cannot effectively reduce interactions between protein molecules. For example, propylene glycol is a plasticizer with weak polarity. Even though it has very small molecular weight, its plasticization efficiency is relatively poor [196].

Generally, a plasticizer interacts with polymer chainsegments to stop crosslinking and thus give a membrane material good ductility and flexibility [199, 207]. Plasticization occurs in the amorphous, high-mobility region. The ability of the plasticizer to prevent hydrogen bonding between molecules is determined by its type and quantity. The glassy state conversion temperature is the important temperature at which the membrane material softens, and chain segments start to move and become rubbery. In semi-crystalline polymers, the melting temperature and crystallinity enhance the softening temperature. When the glassy state conversion and melting temperatures are lower than the degradation temperature by a certain margin, hot plastic formation can be used. This has broad applicationsto membrane packaging. Adding a plasticizer such as moisture or glycerol can weaken the glassy state conversion [202]. Such phenomena can be observed during extrusion of corn starch for preparation of membrane materials with moisture and glycerol as the plasticizers [208]. The starch membrane material recrystallizes if the storage temperature is higher than the glassy state conversion temperature
Chapter 2: Literature Review

[209]. Adding a plasticizer to the membrane material promotes the motion of polysaccharide molecular chains and hence improves its ductility. However, excess plasticizer can impair the effect of condensation between the polysaccharide molecular chains, enhance interaction between plasticizer molecules, and lead to phase separation. Bergo and his partners [210] reported two glassy state conversions of cassava starch membranes, primarily due to the formation of separate phases with high starch and plasticizer contents [211]. Since the starch membrane recrystallizes during placement, its physical and chemical functionalities change with the placement duration [212-213]. Generally, starch-based membrane materials become harder and more brittle after several weeks of placement [8].

However, a study conducted by Mali et al. [214] showed that cassava, corn, and sweet potato starch membranes containing 20% glycerol see their elongation at break values decrease with the incubation time, while their moduli and mechanical strengths exhibit no significant changes. Their study showed that the crystallinities of starch membranes increase with the placement time. The membrane material that contains glycerol recrystallizes more slowly because the plasticizer can interact with polymer chain segments to limit recrystallization. Yet, there are studies which show that a higher plasticizer content promotes polymer recrystallization to a certain extent, and hence increases crystallinity. From a macro-perspective, the membrane material still exhibited lower rigidity and increased flexibility [215].
3. Experimental

3.1 Dynamic Thermomechanical Analysis

Dynamic thermomechanical analysis (DMTA) is a technique to measure how the viscoelasticity of a moulding under a certain oscillation stress changes with a controlled temperature program. It is used to investigate under the action of periodically alternating stress, how material stress–strain relation changes with time, temperature and frequency. A high-molecular weight polymer is a viscoelastic substance and when a periodical alternating stress is applied to it, its elastic and viscous components will change in response to changes in temperature, respectively. Because analyzing the dynamic thermomechanical properties of polymers makes it possible to predict application performance. DMTA is sensitive to vitrifying conversion, crosslinking, crystallization, phase separation and molecular motions of various levels in molecular chain segments. DMTA is suitable method to study the behaviors of molecule motions of polymers. The temperature scanning mode of DMTA is used to detect phase conversions including vitrification.

3.2 Tensile Mechanical Characterisation

The tensile mechanical property test is to apply a force in the direction of the vertical axis to a specimen at the required humidity, strain rate and temperature until the specimen is broken. The force applied to specimens and the specimen deformation are recorded to obtain a tensile stress–strain curve in a stretching orientation. From such tensile stress–strain curves, the tensile properties of materials, such as tensile modulus, tensile strength and elongation at, are obtained. These data are used to evaluate the tensile performance of high-molecular weight materials and thus provide parameters for application specification.

The stress–strain curve of materials includes: an elastic deformation zone and a plastic deformation zone. In the elastic deformation zone, the elastic deformation is reversible, the
stress and strain is in a linear relation satisfying the Hooke Law. In the plastic deformation zone irreversible plastic deformation will occur, and in this case the stress and strain is not in linear proportion and finally the bar specimens with break.

Material tensile strength (MPa) is calculated with the formula 2-1:

\[ \sigma_t = \frac{p}{bd} \] ............................................... (2-1)

where, \( p \) is the load at break, in N; \( b \) is the width of the rectangular part of test specimens, in mm; \( d \) is the thickness of the rectangular part of test specimens, in mm.

Material elongation at break (%) is calculated with the formula 2-2:

\[ \varepsilon_t = \frac{(L-L_0)}{L_1} \] ............................................... (2-2)

where, \( L \) is the distance between reticles of test specimens at break, in mm; \( L_0 \) is the original gauge length; \( L_1 \) is the length of the rectangular part.

Material tensile modulus (MPa) is calculated with the formula 2-3:

\[ \sigma_s = \frac{\Delta f}{L-L_0} \] ............................................... (2-3)

where, \( \Delta f \) is the stress change between two strain values \( (L \) and \( L_0) \) per unit area, in N; \( L \) is the distance between reticles of test specimens at break, in mm; \( L_0 \) is the original gauge length, in mm; \( L_1 \) is the length of the rectangular part of bar specimens.

### 3.3 Wide Angle X-ray Scattering

Wide angle X-ray scattering (WAXS) can be used to analyze arrangement of crystal structures, i.e. at the scale of atoms. When an X-ray beam is impinged on an atomic plane whose lattice planar interval is \( d \) at a swept angle \( \theta \) (the complementary angle to the angle of incidence) and when the angle satisfies the Bragg Law, the diffracted ray enhanced from superposition will be generated in the direction of reflection. Reflection will take place on every reflective plane whose angle \( \theta \) satisfies Bragg Law; after \( \theta \) is measured, the lattice planar interval, the type of
unit cell and size can be determined using the Bragg equation. Furthermore, the atomic arrangement can be determined within unit cells using the intensity of the diffracted ray.

### 3.4 Small Angle X-ray Scattering

Small angle X-ray scattering (SAXS) can be applied to study scattering of particles at a small angle close to the incident X-ray beam. When an X-ray beam is transmitted through a material, if there exists a uneven electron-density distribution at the micro-scale within a material, X-ray scattering will emerge within a small angle close to the incident X-ray beam and SAXS is, in physical nature, the result of differences in electron cloud density between scatterers and surrounding media. SAXS is a powerful technique to study nano-scale liquid and solid structures, SAXS can be used to characterise structures of substances with large unit cells and shape, size and distribution of hyperfine powder particles or hyperfine microvoids of solid substances, less than tens of nanometers. For high-molecular weight materials, SAXS can be used to measure the shape and size of high-molecular weight particles or voids, analyze phase structure, the degree of branched chains and long period of blended polymers. Analysis of SAXS charts allows for quantitative analysis and quantitative calculation. Quantitative analysis includes inhomogeneity of electron cloud density, dispersity of scatterers, monodispersity or polydispersity, to be determined with a Guinier correlation, the sharpness of a two-phase interface (negative deviation from the theorems of Porod or Debye), homogeneity of single-phase electron density (positive deviation from the theorems of Porod or Debye), self-similarity of scatterers (whether to have fractal characteristic) of materials. Quantitative calculation is used to obtain parameters such as scatterer size distribution, radius of gyration, mean scale, related distances, scatterer volume fraction, mean layer thickness, specific surface, mean interface-layer thickness and fractal dimension.\[251\]
3.5 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a type of molecular absorption spectrum, also called molecular vibration spectroscopy. FTIR is used for quantitative and qualitative analysis, and the characteristic absorption peaks of molecules can be used to identify functional groups and chemical structures of these molecules.

3.6 Attenuated Total Reflectance Spectroscopy

Attenuated total reflectance (ATR) FTIR (ATR-FTIR) spectroscopy refers to a technique in which test specimens are affixed on a prism of a material with high refraction index such as AgCl, TlI (KRS-5) or Ge, the angle of incidence is so adjusted that the incident light is totally reflected though it penetrates into the test specimen a few microns. When the test specimen absorbs infrared light at a characteristic wavelengths, the infrared light of that wavelength reflected by the prism is attenuated in intensity. The intermolecular interactions and chemical reactions from mixing of different substances can be presented in a peak change in the spectrum. The ATR-FTIR spectroscopy technique is used to determine infrared spectra of the surface of materials without interference from the prism to provide information about structure of the surface layer of less than 5 μm.

3.7 Scanning Electronic Microscopy

Scanning electronic microscopy (SEM) utilizes a high-energy electron beam to be focused on a specimen surface for point-by-point raster scanning, and the incident electrons interact with the material surface to generate various physical signals that are received, amplified and finally converted by detectors to modulated signals to be displayed on the screen as images of various characteristics of material surface. The re-emission of electrons as a result of interaction between electron beams and materials is used to generate images showing the topography of surface-amplified specimens in the order of time sequence, i.e. the point-by-point imaging
method. Because secondary electrons come from a specimen surface of 5 nm ~10 nm, signal intensity is sensitive to the orientation of micro-regions on the material surface relative to incident electron beams, and more secondary electrons are generated with increasing angle between the specimen surface and the incident electron beam. The image of secondary electrons is useful to display contrast of the topography of specimen surface. The image of secondary electrons is high in resolution, about 3 nm – 6 nm, depending mainly on the diameter of the beam. Resolution achieved in practice, however, is limited by factors such as specimen properties, the method used to prepare specimens and the operational conditions of the microscope (such as scanning speed, beam intensity, specimen inclination, working distance).

### 3.8 Thermogravimetry

Thermogravimetry (TGA) is to measure the relation of mass of materials versus time or temperature under a temperature controlled program. By analyzing the thermogravimetric curves, the loss in mass of tested materials due to temperature is calculated. The thermogravimetric analyzer can be used measure physical phenomena of substances, for instance, evaporation, sublimation, and chemical phenomena such as dehydration, oxidization, reduction, dissociation and degradation. Information about, for instance, the mass loss of materials, whether the mass loss occurs in a single stage or in several stages, the onset/endset temperature of thermal mass loss, and the rate of mass loss, are derived from the thermogravimetric curve (TGA curve). The curve resulting from a first-order derivative TGA curve over temperature or time is called derivative thermogravimetry (DTG) curve that exhibits how the rate of mass loss of a material changes with temperature or time, and it can provide the temperature at which the thermogravimetric mass loss is at the maximum rate.
4. Compatibility and Phase Transitions of Gelatin–HPS Blends

4.1 Introduction

Compatibility of polymers in a blend imparts a significant influence on the final properties of the blend and can lead to complex morphology. Miscibility of two materials depends on whether the free energy of the mixture is negative during the mixing process.

\[ \Delta G = \Delta H - T \Delta S; \text{ requires } \Delta G < 0. \]  

For the mixing process of polymers, mixing entropy change is small due to the long macromolecules, and generally polymer–polymer mixing processes are endothermic, which means \( \Delta H \) is normally positive. Therefore, it is difficult to achieve \( \Delta G < 0 \). As \( \Delta G \) tends to be positive, most polymer blends become immiscible or cannot achieve molecular level mixing, and form heterogeneous systems. In this blend system, gelatin is a protein, hydroxypropyl starch is a water-soluble polysaccharide. Different chemical functional groups are contained in this blended system. Previous studies showed that there is a degree of compatibility, although this requires addition of a compatibiliser with affinity for both blend components.

In this chapter, blends of gelatin with up to 50% hydroxypropylated high amylose (80%) corn starch were developed. Poly(ethylene glycol) (PEG) was used as both a plasticizer and a compatibilizer in the blends. The solutions, films and capsules of the different gelatin–starch blends were characterized by viscosity, transparency, tensile testing, water contact angle and SEM. The linear microstructure of the high amylose starch, and the flexible and more hydrophilic hydroxylpropyl groups grafted onto the starch improved the compatibility between the gelatin and starch.
4.2 Experimental

4.2.1. Materials and solution preparation

A commercially available gelatin (GELITA UG719-N, Sweden) was used in this work. A food-grade hydroxypropylated high amylose (80%) corn starch (A1081) with MS (molar substitution) 0.11 was supplied by Penford (Australia). Poly(ethylene glycol) (PEG 400) was purchased from Sigma. Solutions were prepared using blends of gelatin and hydroxypropylated starch with added plasticizers (water and PEG). Solutions were prepared with different ratios of gelatin and hydroxypropylated starch (100:0, 90:10, 80:20, 70:30, 60:40, 50:50) based on a total weight basis (150 g) including 5%·w/w PEG in 350 mL distilled water. The mixed materials were dissolved in distilled water at 80°C for an initial 30 min at a slow stirring speed (100 rpm), then for a further 30 min at high speed (700 rpm) until a clear solution was obtained. A previous study [291] showed that the gelatinization of this hydroxypropylated starch occurred at about 57°C, which is lower than the temperature of solution preparation used in this study.

4.2.2. Viscosity measurements

The viscosity of the gelatin-starch solutions was measured at room temperature (23°C) using a Brookfield digital viscometer (Model DV-II+ PRO with LV S6-3 spindle) operating at 30 rpm for all blends. The viscometer spindle was immersed into the solution for about 3 min to achieve thermal equilibrium between the solution and spindle with continued shearing. Five viscosity readings were recorded for each solution, and average values were taken. Tests were performed in triplicate.
4.2.3. Casting films and capsule preparation

After degassing, 50 mL of a solution was poured onto a polyethylene plate (10 cm × 15 cm), which was kept level to control film thickness. The cast film was dried overnight at 37 °C, similar to capsule preparation. The dry films were peeled from the plate, placed in a desiccator containing saturated sodium bromide (NaBr) solution, and stored at 56 % RH and 23 °C until required for analysis. Separate control films of pure gelatin and pure starch were prepared in the same way. The weight of the dry films was measured daily until no further measurable weight change was observed, and the thickness of the films was recorded using a micrometer. All films were about 0.3 mm thick with about 8 % SD. Capsules were prepared by dipping stainless steel mold pins (cylindrical, 7 mm diameter) into the solutions and then drying at 37 °C, as described in detail previously [121]. Drying time depended on capsule rigidity, and those containing a higher concentration of starch required a longer drying time, as starch has a stronger hydroxyl bond with water than gelatin. The drying time was increased gradually from 30 to 50 min with increasing starch content from 0 to 50 %. Processability was determined by evaluating the viscosity and gelatinisation temperature of the various blends. An infrared heating balance (Model DHS-20) was used to measure moisture content in blends through heating them to 110 °C for 20 min.

4.2.4. Transparency measurements

A UV (WFZ UV-3802) spectrum was used to measure the transparency of the different solutions, which were placed in a 10 mm × 10 mm square sample container for measurement. A wavelength of 206 nm was used to indicate transparency in this work. The transparency of different films was also measured at a wavelength of 206 nm and divided by film thickness and presented as %/mm.
4.2.5. Mechanical properties

Dumbbell-shaped specimens (gap 50 mm, width 1 mm) were cut from cast films then equilibrated at 56 % RH (controlled by NaBr solution) for 72 h before testing. The tensile properties of specimens were measured in accordance with ASTM D638 using an Instron mechanical testing apparatus (Model 3366). The Young modulus, tensile strength and elongation at break were measured at a crosshead speed of 10 mm/min. Each test trial per film consisted of seven replicate measurements.

4.2.6. Contact angle measurement

The water contact angles of the different films were measured at room temperature (23 °C) using an FTA 200 goniometer (first ten angstroms). Measurement was carried out immediately after dropping the water (0.1 mL) onto surface to avoid the effects of receding droplet.

4.2.7. Scanning electron microscopy (SEM)

A Phillips XL-30 FEGSEM scanning electronic microscope (SEM) was used to investigate the surfaces of the different films. Specimens were first coated with iridium to a thickness of ∼0.2 µm in a vacuum evaporator using a Sputter Coater (POLARON SC5750), and subsequently viewed in the SEM at a low accelerating voltage of 2 kV.
4.3 3. Results and discussions

4.3.1. Transparency of solutions

Photographs of the solutions with different gelatin–starch ratios are shown in Figure 4-1. No phase separation was observed in the individual solutions. While the pure gelatin solution is reasonably clear, the pure starch solution is cloudy and opaque, due to the retrogradation of starch, which had already been significantly decreased through hydroxypropylation [92, 113]. The results of the UV spectra measurements of the transparency of the different solutions are shown in Figure 4-2, and it is seen that the transparency ratio decreased gradually with increasing starch content. It is noted that the transparency of the pure starch solution was lower than that of all blended solutions, which confirms that the cloudiness of blends is due to starch, and not phase separation. The addition of PEG increased the transparency of the blends, indicating improved compatibility between gelatin and starch.
Figure 4-2 Transparency of the solutions with different gelatin–starch contents

4.3.2. Viscosity of solutions

Figure 4-3 Viscosity of the various gelatin–starch blends under the same stirring rate (30 rpm)

Figure 4-3 shows the viscosities of the solutions of the different gelatin–starch blends under the same stirring rate and at the same temperature. It is seen that viscosity increased significantly with increasing starch content, which is expected since viscosity of the starch solution was much higher than that of gelatin. Gelation temperature, or more particularly the time to the onset of gelation, is of critical importance in many applications of gelatin-based materials,
including hard capsule manufacture. Despite this, there is no universally accepted or adopted procedure for measuring the setting time [138], even though various methods have been developed. These methods are mainly based on detecting either the time at which the viscosity of the solution increases sharply, or a particular degree of rigidity after the setting point has been passed [121, 138]. In this work, we focused on the effect of temperature and incubation time on viscosity.

![Figure 4-4](image_url)

Figure 4-4 Effect of incubation time at 60 °C on the viscosity of gelatin–starch blends: (A) 100:0; (B) 90:10; (C) 80:20; (D) 70:30; (E) 60:40; (F) 50:50

Figure 4-4 shows the effect of incubation time at 60 °C on the viscosity of the different gelatin–starch blends. The viscosity of the pure gelatin decreased during the first 3 h of incubation then remained stable, as reported previously [138]. For the blended solutions, the decrease ratio with time became less pronounced with increasing starch content, which could be simply explained by the higher viscosity of the solutions containing starch. Figure 4-5 shows the effect of temperature on the viscosity of the solutions with different gelatin–starch contents. As expected, the viscosity of all blends increased with decreasing temperature. It should be noted that there is an inflection in the viscosity curve for all blends at about 50–60 °C. This inflection is usually used to indicate the onset of gelation in hard capsule manufacture. The
onset temperature can be clearly seen for all blends, although it increased and the slope of the onset became less steep with increasing starch content.

![Figure 4-5](image)

Figure 4-5 Effect of temperature on the viscosity of gelatin–starch blends

### 4.3.3. Properties of capsules and films

![Figure 4-6](image)

Figure 4-6 Photos of capsules made from solutions with different gelatin/starch contents: (A) 100:0; (B) 90:10; (C) 80:20; (D) 70:30; (E) 60:40; (F) 50:50

Photographs of capsules made from the solutions with different gelatin–starch content are shown in Figure 4-6. It is seen that the transparency of the capsules decreased with increasing starch content, corresponding with the transparency of the solutions (see Figs. 4-1 and 4-2). Capsule wall thickness increased with increasing starch content under the same dipping conditions, since the starch increased the viscosity of the solutions. In practical terms, the total
solids concentration in blended solutions should be decreased with increasing starch concentration to reduce the viscosity.

The mechanical properties of the different films were studied by tensile testing, and the results are shown in Figure 4-7. It is seen that the Young modulus gradually increased with increasing starch content up to 50%. Tensile strength increased slightly, especially for lower starch content films. The elongation of the films gradually decreased with increasing starch content. Generally, the films became more rigid and brittle with increasing starch content after equilibration under the same humidity conditions. Similar results for fish gelatin–sago-starch blends have recently been reported [232]. It is noted that PEG increased the elongation of the blends indicating decreased brittleness.

4.3.4. Microstructures and phase composition of different blends

SEM was used to investigate the microstructures of the surfaces of the films made from the various blends (see Figure 4-8). Some protrusions were observed on the surfaces of the films containing starch, and the density of these protrusions increased with increasing starch content. This phenomenon indicates that gelatin and starch are two phases, and that the shrinking ratios of gelatin and starch are different during drying. The individual surfaces are generally present as a continuous phase without indication of phase separation, which means the gelatin and starch are compatible although they are immiscible. Table 4-1 lists the water contact angles of the different films, and it is seen that the films of pure gelatin and the blends
containing up to 50% starch have similar water contact angles. The results indicate that gelatin is a continuous phase, while starch is a separated phase distributed in gelatin and covered by gelatin in all the blends. The starch has smaller water contact angle than gelatin indicating that the starch is more hydrophilic. The better hydrophilic properties guarantee that the blended materials have good water solubility, which is important for capsule materials.

Table 4-1 Water content and contact angles of various blends

<table>
<thead>
<tr>
<th>Gelatin–starch ratio</th>
<th>100:0</th>
<th>90:10</th>
<th>80:20</th>
<th>70:30</th>
<th>60:40</th>
<th>50:50</th>
<th>0:100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content (%)</td>
<td>17.2 ± 2.1</td>
<td>17.5 ± 2.6</td>
<td>17.4 ± 1.9</td>
<td>17.8 ± 2.4</td>
<td>18.1 ± 2.3</td>
<td>17.8 ± 2.1</td>
<td>18.5 ± 2.2</td>
</tr>
<tr>
<td>Contact angle (°)</td>
<td>113.7 ± 4.1</td>
<td>111.9 ± 2.6</td>
<td>110.7 ± 1.9</td>
<td>111.3 ± 2.1</td>
<td>107.7 ± 1.8</td>
<td>106.3 ± 3.4</td>
<td>72.3 ± 5.8</td>
</tr>
</tbody>
</table>

Although phase separation has been widely reported for many gelatin–starch blends, their compatibility can be improved through various processing methods, as previously mentioned. A possible explanation for the improved compatibility found here is the linear microstructure of the high amylose starch, and the flexible and more hydrophilic hydroxypropyl group grafted onto the starch. Using differential thermal analysis (DTA) [177, 293] have also observed this non-phase separation phenomenon in gelatin–hydroxypropylated potato starch in a low concentration (2–3%) solution. Further-more, the addition of PEG is expected to improve the compatibility between gelatin and starch, as it can be dissolved well into both components [124, 205, 293]. Miscibility of an immiscible polymer blend can be improved by a compatibilizer, i.e. any polymeric interfacial agent that facilitates the formation of uniform blends. PEG acted as both a plasticizer and a compatibilizer in the gelatin–starch blends.
studied here, as indicated by improved transparency and decreased brittleness. The compatible microstructure and continuous phase of the blends containing up to 50% starch enabled the production of reasonably good films and capsules. The issue of compatibility and miscibility in this blends will be studied in detail in subsequent chapters.

![Image of film surfaces of various blends observed under SEM](image)

Figure 4.8: The film surfaces of various blends observed under SEM

### 4.4 Conclusions

Blends of gelatin with up to 50% hydroxypropylated high amylose corn starch have been developed as hard capsule materials. The use of poly(ethylene glycol) (PEG) as both a
plasticizer and a compatibilizer increased the transparency and toughness of the various blends. The viscosity of the solutions increased significantly with increasing starch content. The onset temperature of gelation was evident for all solutions, and it increased and the slope of the onset became less pronounced with increasing starch content. The linear microstructure of the high amylose starch, and the flexible and more hydrophilic hydroxylpropyl group grafted onto the starch improved the compatibility between the gelatin and starch, although they remained immiscible. The addition of PEG improved the compatibility between the gelatin and starch, as it dissolved well into both. All film surfaces presented a continuous phase (gelatin) confirming that gelatin and starch are compatible. The compatible microstructure and continuous phase in blends containing up to 50% starch enabled the production of reasonably good films and capsules.
5. Phase Composition and Interface of Starch–Gelatin Blends

5.1 Introduction

Phase composition and interface of a polymer blend are important and attract much scientific attention since they influence processing behavior and performance of blends, in particular for immiscible blended systems. For various reasons, developing edible films by blending starch with gelatin has attracted much attention. For example, a blended film of polysaccharides and proteins shows better gas barrier (O\(_2\) and CO\(_2\)) than any pure film [74, 177, 197, 293]. Previous research has shown that gelatin and starch are immiscible [59, 146], however, their morphologies and compatibility are affected by various factors, such as processing time [131], temperature [177], pH [81, 166] and solid concentration [97].

Like other immiscible blends, rheological and mechanical properties of starch–gelatin blends depend on their morphology, particularly in terms of their degree of homogeneity and the composition of their continuous and dispersed phases. Various techniques have been used to study phase separation of this complex blended system, including differential scanning calorimetry (DSC) [125, 150], dynamic mechanical thermal analysis (DMTA) [148, 171], rheometry [73, 91, 159], polarized optical microscopy [289] and confocal light scanning microscopy (CLSM) [130]. Fourier transform infrared (FTIR) spectroscopy with a microscope provides a capability to combine optical microscopy image analysis with chemical analysis via FTIR spectroscopy for evaluating polymer blends [283, 284]. Investigation of composition and interface of a blend using FTIR micro-spectroscopy enables unique insight into the interface and morphologies of the system since it is based on chemical contrast between constituents. For example, a FTIR micro-spectrometer to study the composition of gelatin–amylopectin blends prepared by extrusion [284]. FTIR two-dimensional maps were obtained based on the ratio of
the peak areas of saccharide and amide bands. The role of the integrity of the starch granule in defining composition fluctuations that could control the performance of these blends was investigated. However, there is no report about the phase composition of film from solution that are expected to have more homogeneous structure. Furthermore, investigation on the interface of starch–gelatin blends, in particular the polymer chain diffusion, could further explore the mechanisms of compatibility of the blend system.

The mapping resolution depends on the size of the detecting region. Theoretically an FTIR beam is concentrated in a small region, but that will result in lower sensitivity for a normal FTIR instrument. Synchrotron FTIR has a much higher signal-to-noise (S/N) ratio and higher spatial resolution, which allows mapping of the microstructure, as well as providing insight into the chemical distribution and interactions [283]. It can provide a effective technique to complement other techniques for investigating starch and/or gelatin phases in the blends, and in particular chemical composition.

In this chapter, gelatin and hydroxypropylated high amylose (80%) corn starch were blended with poly(ethylene glycol) (PEG) as plasticizer through a solution method. An objective was to develop gelatin–starch blends for use in making drug capsules by well-established technology of dipping stainless mold into solution and then drying [267]. Equilibrated solutions will be studied in this work. A synchrotron FTIR with micro-spectroscopy facility will be used to study the interface and phase composition of gelatin–starch blends of cast films. The contribution of PEG on the morphology of the blends was investigated based on the mapped composition.

5.2 Experimental

5.2.1. Materials and solution preparation

A commercially available gelatin (GELITA UG719-N, Sweden) was used in this work. A food-grade hydroxypropylated high amylose (80%) corn starch (A939) with MS (molar
substitution) 0.11 was supplied by Penford (Australia). Poly(ethylene glycol) (PEG 400) was purchased from SigmaAldrich.

20 % water solutions were initially prepared for various characterizations. Solutions were prepared with different ratios of starch–gelatin (100:0, 80:20, 60:40, 40:60, 80:20, 0:100) based on a total weight basis (100 g) in 400 mL distilled water. The mixed materials were dissolved in distilled water at 80 °C for an initial 30 min at a slow stirring speed (100 rpm), then for a further 30 min at high speed (700 rpm) until a clear solution was obtained. Previous studies (LAN Et Al., 2010; Liu et al., 2010) showed that the gelatinization of this hydroxypropylated starch occurred at about 57 °C, which is lower than the temperature of solution preparation used in this study. Solutions containing 5 % PEG were prepared by the same method.

5.2.2. Film and specimen preparations

Low concentration solutions were used to prepare very thin cast films to be used for transmission microscopy. The solutions described above were diluted with water to 2 % concentration and stirred at a speed of 500 rpm for 10 min. After degassing, 5 mL of solution was poured onto a PET dish (diameter 5 cm), which was kept level to control film thickness. The cast film was dried overnight at 37 °C. The dry films were peeled from the plate, placed in a desiccator containing saturated sodium bromide (NaBr) solution, and stored at 56% RH and 23 °C until required for analysis. All films were about 5–7 μm in thickness.
The blend used for studying interfaces was prepared using a highly concentrated solution (20% described above. The pure starch solution was first added into a small plastic container, and then the pure gelatin solution was added on top of the starch solution at room temperature. Since the viscosities of both solutions were very high they did not mix together without stirring. After drying slowly at room temperature, a specimen with two layers of material was removed from the container. The specimen was embedded into epoxy resin so that vertical cross-section was exposed. A thin cross-section film (about 7–9 μm) with two parts of materials (see Figure 5-1) was cut from the blend using a microtome. All the films were scanned with FTIR-ATR (Bruker Tensor 37) first to search for characteristic peaks of starch or gelatin.

5.2.3. Synchrotron Fourier transform infrared micro-spectroscopy (Synchro-FTIRM)

The specimens were analysed using a Bruker Vertex V80v Fourier transform infrared spectrometer coupled with a Hyperion 2000 microscope equipped with a liquid nitrogen cooled narrow-band mercury cadmium telluride (MCT) detector at the Australian Synchrotron infrared beamline. The high brilliance of synchrotron radiation–IR allowed an aperture of 5 μm × 5 μm to achieve a high S/N ratio and high spatial resolution over the 140 x 140 μm image field. This
allowed high quality mid-IR spectra to be achieved with a relatively low number of scans. Spectral collection was made in transmission mode at 4 cm\(^{-1}\) resolution, 32 scans were co-added and converted to absorbance using OPUS 6.5 software.

5.2.4. Analysis of the spectra

For all spectra, the integration area of the saccharide bands (1180–953 cm\(^{-1}\)), which is labelled as Band-1, was used to represent starch. The integration area of the amide I and II bands (1750–1483 cm\(^{-1}\)), labelled as Band-2, was used to represent gelatin. Band-1 divided by Band-2 was used to evaluate the relative starch content during analysing mapped images. OPUS 6.5 was used to reconstruct the 3D image using the ratio of integrated areas of starch and gelatin.

For a more detailed analysis, the corresponding spectral data were reconstructed with CytoSpec 1.4 (CytoSpec Inc., New York, USA) software to obtain chemical maps of the ratios of integrated areas under starch and gelatin peaks. Images and maps were contrasted using the Jet color scheme available in CytoSpec, with red indicating the highest relative concentration of starch, while blue indicated the highest relative concentration of gelatin.

5.3 Results and Discussion

Figure 5-2 shows typical transmission FTIR spectra of a starch and gelatin film acquired using the FTIR microscope with an aperture size of 5 μm × 5 μm, respectively. The distinctive spectral features for starch were CO and CC vibrational modes that are highly coupled from 1300 to 800 cm\(^{-1}\), which are sensitive to conformational and crystalline order of starch [283, 284]. The absorption bands at approximately 1155, 1125, and 1105 cm\(^{-1}\) due to CO, CC stretching with some COH contributions, while 1080, 1047, 1022, 995, and 928 cm\(^{-1}\) belong to COH bending and CH\(_2\) related modes. For the starch, a series of overlapping peaks located in the region of 1180–953 cm\(^{-1}\), were the most intense bands in the mid-IR spectrum. Furthermore,
3400–3100, 3000–2700 cm\(^{-1}\) belong to OH and CH stretching vibrations, respectively. The typical spectral features for the protein were strong amide I and II bands located at approximately at 1650 and 1540 cm\(^{-1}\), respectively. The amide I absorption was primarily due to the stretching vibration of the C=O bond and the amide II band was due to the coupling of the bending of the N-H bond and the stretching of the C-N bond.

Figure 5-2 Transmission FTIR of thin films of pre-gelatinized starch, gelatin, and a 2:1 starch–gelatin blend

Figure 5-2 shows a typical FTIR-ATR spectra of starch–gelatin (2:1) blend. The bands associated with individual components in addition to the contributions of the water absorptions at 3300 cm\(^{-1}\) (OH stretching), 1630 cm\(^{-1}\) (COH bending), with a broad combination band centered around 2200 cm\(^{-1}\). The bands for starch and gelatin were identified in the spectra and no new bands were detected, which means it is a phase separated system. In order to characterize the starch or gelatin content quantitatively, the integrated area of the saccharide bands (1180–953 cm\(^{-1}\)), labeled as Band-1, was used to represent starch; while the integrated area of the amide I and II bands (1740–1486 cm\(^{-1}\)), labelled as Band-2, was used to represent gelatin. The Band-1 divided by Band-2 was used to evaluate the starch content during analysis.
of mapped images, which is effective in eliminating the absorbed water and effects due to the blend itself.

Fig 5-3 Integrated plot of the Bands-1/Bands-2 ratios

To quantitatively study the relationship between the ratio of band areas and concentration functions, the ratio of the area of the starch bands (Band-1) to that of gelatin bands (Band-2) for each blend composition was shown in Fig 5-3. The ratio of Band-1 to Band-2 increased with increasing starch content, though not in a linear correlation. Based on the differences between starch and gelatin in the FTIR spectral region, the distribution of starch or gelatin in the blends could be obtained from a 3-D contour map (see Fig 5-5) that relates the ratio of the peak area of the starch bands to that of gelatin bands. The ratio of band areas rather than absolute values was used to enable normalization of the results, correcting for possible variations in thickness both within the same film and between different films.
Figure 5-4 shows the transmission FTIR spectrum scanned across the interface of a film containing two materials (corresponding with Figure 5-1). The bands on both starch and gelatin sides were identified and shown as smooth continuous signal phases. It is interesting to note that there is a layer of about 7–9 μm thickness, in which both Band-1 and Band-2 were detected, indicating that gelatin and starch were co-existent. The result means starch and gelatin are compatible, but not miscible; the polymer chains diffused into each phase at the interface. The starch used in this work has a high proportion of flexible linear amylose chains, with a more hydrophilic hydroxypropylene chain improving the compatibility with gelatin and retarding retrogradation.
Fig 5-5 shows variation of the ratio of the area of the Band-1/Band-2 plotted as 3D contour maps for various starch–gelatin blends. This image was generated from the data of single measurements by allocating a colour to each pixel based on the ratio of Band-1 and Band-2, which was used to characterize the starch–gelatin content. In the 3D contour maps for various starch–gelatin blends, the Y-axis show the ratios of Band-1/Band-2, which means that the relative content of starch increased with increasing Y-axis value. Similarly, the monocolour optical images represent the ratio of various starch–gelatin blends, with darkness reflecting the content of starch distribution. From the 3D images, the red peak in the scale denotes a high value of starch while the blue bottom region denotes a high value of gelatin. G40/S60 is a typical count contour map for starch–gelatin 40:60 blend (Fig 5-5). It was observed that the starch phase (Band-1) distribution was a dispersed phase, while gelatin phase (Band-2) was a
continuous phase. However the sharp peak, not the column, of Band-1 indicates there are certain chain diffusions across the interface of starch domains. That means the complete demixing of starch and gelatin domains did not occur, which corresponded with the results of scanned interfaces (Fig 5-1). From Fig 5-5, it can be seen that starch was a dispersed phase in various starch–gelatin blends, and the size of starch domains increased with increasing starch content, which is similar to the results from optical images.

To quantitatively analyse the chemical maps of theratios of integrated areas under starch and gelatin bands, the 2D intensity contour maps were established for blends with different starch–gelatin contents. Figure 5-6 shows the chemical map of starch distribution acquired from the MCT detector for the various starch–gelatin blends. The colour code represents the concentration of a component. The red in the scale denotes a high value of starch while blue
denotes a high value of gelatin. The FTIR microscopy images confirmed that the starch phase distribution was a dispersed phase. The size of starch domains increased with increasing starch content.

The FTIR spectrum concentration maps suggested that for all mixtures investigated gelatin formed a continuous matrix in which starch inclusions were dispersed. The results demonstrated the highly heterogeneous nature of such blends with starch domains dispersed into a gelatin continuous phase even with higher starch content blends. All FTIR spectra showed contributions from both starch and gelatin absorptions and therefore indicated that complete demixing, with pure starch and gelatin domains, did not occur. The FTIR spectral results support the observation that starch and gelatin are partly compatible.

Figure 5-7 Contour plots of the variation of the Band-1/Band-2 ratio for different starch–gelatin blends: (a) 60:40; (b) 40:60; and with 5 % PEG: (A) 40:60; (B) 60:40
Figure 5-7 shows contour plots for variation of the Band-1/Band-2 ratio for the starch–gelatin blends with and without PEG. It is seen that the ratio of Band-1 was increased significantly after addition of PEG, even only 5%. The increase of the Band-1 was contributed by PEG resulting in an improvement of the interface between starch and gelatin. Figure 5-8 shows the FTIR-ATR spectra of PEG and starch containing PEG. It is noted that there are some bands for PEG [245], just in the range 1180–953 cm$^{-1}$ that overlap with Band-1 used for representing starch. The overlap of the bands of PEG and starch enhanced the intensity of the Band-1 in the maps, resulting in enhanced color of Band-1 and enlarging its area. It is noted that the blue colored area (Band-2) became weaker after adding PEG, indicating the PEG could dissolve homogeneously into gelatin. PEG is miscible with starch [205, 245, 293]. It is expected that PEG at the inter-phase between starch and gelatin improved their compatibility. The improvement of the compatibility or interface enlarged the area of Band-1.
Figure 5-8 Contour plots of the variation of the Band-1/Band-2 ratio plotted as 3D for different starch–gelatin blends: a) 60:40; b) 40:60; and with 5% PEG: A) 40:60; B) 60:40.

5.4 Conclusions

Interfacial and phase composition of various starch–gelatin blends were investigated by FTIR spectroscopy with various extended techniques, from scanning across an interface to 2D and 3D mapped by synchrotron FTIR micro-spectroscopy. The peaks of the saccharide bands (1180–953 cm\(^{-1}\)) and the amide I and II bands (1750–1483 cm\(^{-1}\)) were used to identify the starch and gelatin, respectively. The ratio of the areas of the starch and gelatin bands was used to determine the relative distributions of the two components in the blends. The FTIR concentration maps suggested that for all the mixtures investigated, gelatin formed a continuous matrix in which starch inclusions were dispersed. All FTIR spectra showed contributions from both starch and gelatin absorptions and therefore indicated that complete demixing with pure starch and pure gelatin domains did not occur. There was an about 20 \(\mu\)m thickness layer where gelatin and the starch were in co-existence, indicating gelatin and the starch were compatible to a certain degree in this system. The PEG homogeneously distributed in both gelatin and starch phases, and blurred the interface between gelatin and starch, indicating that PEG acted as a plasticizer and as a compatibilizer for the gelatin–starch blends. The starch used in this work has more flexible linear amylose chains, and with the more hydrophilic hydroxypropyl substituents improving the compatibility with gelatin.
6. Phase Inversion and Compatibility studies of Gelatin–HPS Blends

6.1 Introduction

The influence of different processing conditions on the phase distribution and phase inversion of gelatin–HPS blends through observation by microscope are presented in this chapter. It is difficult to observe the topology of blends and the phenomenon of mixing between phases as the contrast of unstained images is poor. By virtue of the principal that starch chain segments would generate an inclusion compound with iodine and consequently develop a color, a method of using iodine to stain the HPS constituent selectively was applied in these studies to observe the constituent distribution in the blended membrane. It is suggested by experiments that this method would achieve a stain in a faster manner without dissolving or damaging the structure of the original membrane, and reveal phase distribution in the blends. Therefore this method brings a simpler, clearer and more illustrative way to research the topology features of gelatin–HPS and the phase distribution phenomenon. This method is of a guiding significance in the aspect of methodology on the phase distribution and phase inversion in other starch-based blends.

The phase distribution of blend membranes under different blending proportions, phase inversion and the compatibility of blends are presented and analysed in this chapter. This research and analysis will provide important and intuitive evidence for research on phase inversion and compatibility that was reported in Chapter 5. In addition, the influence of processing conditions, such as stirring rate, stirring time, density, temperature and incubation time, to the phase distribution and compatibility of the blends were studied and results presented in this chapter. These studies strengthen learning of these blends more comprehensively, and serve as guidance to the production and processing in the future.
6.2 Experimental

6.2.1. Materials and Equipment

A commercially available gelatin (GELITA UG719-N, Sweden) was used in this work. A food-grade hydroxypropylated high amylase (80%) corn starch (A939) with MS0.11 was supplied by Penford (Australia). An Olympus BHZ-UMA microscope, in transmission mode with a magnification of 500X, was used in these observations of membranes.

Preparation of gelatin-HPS blends: Water solutions of 3% were initially prepared for various characterizations. Solutions were prepared with different ratios of gelatin–starch (100:0, 70:30, 50:50, 45:55, 40:60, 30:70 and 0:100) based on a total weight basis (100 g) in 400 mL distilled water. The mixed materials were dissolved in distilled water at 80 °C for an initial 30 min at a slow stirring speed (100 rpm), then for a further 30 min at high speed (700 rpm) until a clear solution was obtained. Previous studies (LAN Et Al., 2010; Liu et al., 2010) showed that the gelatinization of this hydroxypropylated starch occurred at about 57 °C, which is lower than the temperature of solution preparation used in this study. The 5% and 7% water solutions were prepared by the same method.

6.2.2. Influence of blend proportion to the topography of gelatin–HPS blends

Blends with difference proportions of gelatin to HPS(100:0, 70:30, 50:50, 45:55, 40:60, 30:70 and 0:100) were prepared and cast onto glass slide, the glass slide was inclined to leave a thin membrane of solution in the glass slide. Drying the film left a thin membrane under room temperature, the dried membrane was stained with 1% iodine, and the unstained and stained regions were observed using an Olympus BHZ-UMA microscope. The films prepared in this section were plasticized by residual water, about 8-10% w/w, with no other plasticiser added.
6.2.3. Influence of stirring on the topography of gelatin–HPS blends

Blends were processed with different stirring rate (200 rpm, 500 rpm, and 1000 rpm), the detailed methods of preparation and stain shall consist with chapter 6.2.2; observe the stained membranes with an Olympus BHZ-UMA microscope.

Blends were processed with different stirring time (1 h, 3 h, 5 h, 7 h, 17 h), the detailed methods of preparation and stain were consistent with Chapter 6.2.2; observation of the stained membranes was made with an Olympus BHZ-UMA microscope.

6.2.4. Influence of temperature to the topography of gelatin–HPS blends

Blends were processed with varying temperature (20 °C, 40 °C, 60 °C, 80 °C), the detailed methods of preparation and stain were consistent with Chapter 6.2.2; observation of the stained membranes was made with an Olympus BHZ-UMA microscope.

6.2.5. Influence of incubation time to the topography of gelatin–HPS blends

Blends were processed with different incubation time (1 h, 3 h, 5 h, 7 h, 24 h), the detailed methods of preparation and stain were consistent with Chapter 6.2.2; observation of the stained membranes was made with an Olympus BHZ-UMA microscope.

6.3 Results and discussions

6.3.1. Influence of blend proportion on the topography of gelatin–HPS blends

Microscope figures of gelatin–HPS blends are shown in Figure 6-1. The figures in the first row are unstained membranes. Both the pure starch membrane and the gelatin membrane have a relatively smooth surface. The structure of blends is rough, heterogeneous, indicating that gelatin was partially compatible with HPS. It is shown that HPS is a continuous phase, while gelatin is a dispersed phase when the proportion of HPS was 50%. Regarding blends containing 70% HPS, the gelatin serves as a close-packed phase, evenly distributed in the HPS
continuous phase with globular shapes and elliptical shapes with diameter of 50 μm. The continuous phase is gelatin and the dispersed phase is HPS when the proportion of HPS is lower than 50%. However the shape of HPS in the dispersed phase was different with round shapes and olive shapes of gelatin, the shape of HPS in the dispersed phase was irregular long striped structures with branches. The blend with an HPS proportion of 50:50 was the phase inversion composition.

<table>
<thead>
<tr>
<th>Composition: gelatin:starch</th>
<th>Unstained</th>
<th>Stained</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>30:70</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>40:60</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>
### Figure 6-1
Optical images of blends with different proportions (scale bar equals to 80 μm, other images have the same scale)

<table>
<thead>
<tr>
<th>Proportion</th>
<th>Image 1</th>
<th>Image 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>45:55</td>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
</tr>
<tr>
<td>50:50</td>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
</tr>
<tr>
<td>70:30</td>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
</tr>
<tr>
<td>100:0</td>
<td><img src="image_url" alt="Image" /></td>
<td><img src="image_url" alt="Image" /></td>
</tr>
</tbody>
</table>

The figures in the second column are microscopic pictures of stained blends. HPS was coloured purple by iodine, while gelatin was not coloured. The purple regions in the second column represent HPS, and the unstained regions are gelatin. Comparing the unstained images
Chapter 6: Phase Inversion and Compatibility studies of Gelatin–HPS Blends

and coloured images, it is seen that staining by iodine of the membrane did not damage the phase distribution of the membranes; therefore, this method is feasible for visualising the starch without altering the morphology. The phase distribution of HPS in membranes is contrasted in the images of stained specimens. The stained images show that there are many purple spots in the gelatin phase, indicating that much HPS exist in the gelatin phase, with the occurrence of blends between phases, and the blends are partially compatible. Research by Chaleat et al suggests that starch–poly(vinyl alcohol) with thermoplastic features presents a phase separation phenomenon, however blends with complex phases can occur [277]. The pictures for 30:70, 40:60, 45:55 and 50:50 are shown in Figure 6-1, the phase of the blends were inverted as the HPS content changed, the HPS continuous phase gradually became more tenuous as the content of gelatin increased, and the dimensions of the gelatin dispersed phase became increasingly larger. HPS was not able to form a continuous phase and changed into a dispersed phase, which packed within the continuous phase of gelatin in a melange of puddle shapes partially interconnected with random rivulets, when the content of gelatin reached 50 %. In conclusion, the constituent content is of great significance to the phase distribution and phase inversion of blends.

6.3.2. Influence of stirring rate on the topography of gelatin–HPS blends

The phase distribution of blend membranes prepared under difference stirring rates are shown in Figure 6-2. In blends with HPS as a continuous phase, the dispersed phase of gelatin was not consistently different as the stirring rate increased. Assuming both gelatin and starch are dissolved at a particular temperature, then mixing solutions should be independent of stirring speed since the process is not a dispersion that would involve stirring rate. Similarly, when gelatin was the continuous phase, the stirring rate had little distinct influence on the topography of blends, more commingling between phases was observed, indicating that
higher stirring rate will not improve the mixing of solutions, as it would the dispersion of blends.

<table>
<thead>
<tr>
<th>Gelatin: HPS</th>
<th>200rpm</th>
<th>500rpm</th>
<th>1000rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>30:70</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>40:60</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>45:55</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>50:50</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>
6.3.3. Influence of stirring time on the topography of gelatin–HPS blends

Optical micrographs of blend membranes with different stirring time are shown in Figure 6-3. As for 30:70 blends, more inter-mixing between phases was observed as the blending time increased, the interface of gelatin-HPS became blurred, indicating that the dispersion of both phases was improved subject to the prolonged blending time. The topographies of blends of 40:60 and 45:55 exhibited no distinct change as stirring time increased, increased blending between phases occurred. More blending between phases occurred in blends of 50:50 and 70:30 as the stirring time increased, HPS was dispersed in gelatin in a more uniform manner. In conclusion, long blending time improved the dispersion of blends.

<table>
<thead>
<tr>
<th>Gelatin:HPS</th>
<th>1h</th>
<th>3h</th>
<th>5h</th>
<th>7h</th>
<th>17h</th>
</tr>
</thead>
<tbody>
<tr>
<td>30:70</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td>40:60</td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>
6.3.4. Influence of temperature on the topography of gelatin–HPS blends

The topographies of blend membranes formed under ambient temperature on glass slides from solutions equilibrated under different temperatures are shown in Figure 6-4. When the proportion of HPS in blends was above 50 %, solutions with temperature above 40 °C would form network-like structures after dripping on the glass slide under ambient temperature, the higher the temperature, the more compact the network structure became. A reason may be that higher temperature solutions would cool more rapidly when the solution reached the cold surface of a glass slide, and the HPS constituent in the blend would form a fine dispersion of components within the film. More rapid cooling will lead to a spinodal separation from the solution, while slower cooling would cause bimodal or coexistence curve separation. Higher temperature enabled the HPS molecular chains to become more soluble giving solvent expanded coils, with higher film forming capability. For blends with lower than 50 % HPS content, the HPS network structures were formed only when the temperature was above 80 °C. The HPS proportion of 50% was the concentration for phase inversion, when the membrane film was formed under ambient temperature, however as the membrane...
temperature increased, the HPS proportion of 30% become the concentration for phase inversions suggesting that the membrane temperature is of great significance to the phase inversion of blends.

<table>
<thead>
<tr>
<th>Gelatin:HPS</th>
<th>21 °C</th>
<th>40 °C</th>
<th>60 °C</th>
<th>80 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>30:70</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>40:60</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>45:55</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>50:50</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
</tr>
<tr>
<td>70:30</td>
<td><img src="image17.png" alt="Image" /></td>
<td><img src="image18.png" alt="Image" /></td>
<td><img src="image19.png" alt="Image" /></td>
<td><img src="image20.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure 6-4 Optical images of films made by solutions of different temperature (show the same scale level with Figure 6-1)

6.3.5. Influence of incubation time on the topography of gelatin–HPS blends

The topography of membranes prepared by solution from an upper layer of gelatin–HPS blends with different incubation times is shown in Figure 6-5. These figures were used to
observe the phase distribution in blends separated further as the incubation time increased. Blends of 30:70 gelatin:HPS combined with nearby HPS to form structures larger in volume and quantity due to the interfacial tension, as the incubation time increased. HPS regions could combine further as the incubation time increased, their quantity became larger and they precipitated in the solution. HPS in an upper layer dispersed in the dispersed phase in gelatin, rather than the continuous phase after 24 h standing. Similar phenomenon was observed in blends 40:60, HPS finally precipitated after combining with each other, the precipitation occurred after 5 h standing. The HPS in the upper layer dispersed in the droplet phase in the gelatin of the continuous phase after 24 h standing. In blends of 45:55 the HPS in the upper layer started to precipitate after 1 h standing, the upper layer solution formed a joint continuous topography immediately after the phase separation. HPS gradually turned into a dispersed phase structure embedded in droplet shapes over time. No combination in HPS in blends of 50:50 and 70:30 was observed; fork shaped HPS precipitated significantly after standing for 7 h and 5 h separately, HPS dispersed in droplets in the continuous phase of gelatin. However, the size of droplets varied, the size of droplets in HPS phase in 70:30 was the smallest. In conclusion blends of 45:55 precipitated at first, because the HPS interface in blends of 45:55 was the thinnest, the blends were in a non-equilibrium state due to the action of interfacial tension on standing, the HPS interface finally ruptured and precipitation occurred. There are numerous mechanisms involving interfacial tension, which include Rayleigh distortion [278-280], contraction and end-pinching [281, 282], and the disperse state of blends will change with these mechanisms.

<table>
<thead>
<tr>
<th>Gel:HPS</th>
<th>1h</th>
<th>3h</th>
<th>5h</th>
<th>7h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>30:70</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Figure 6-5 Optical images of blends after different incubation time (show the same scale level with Figure 6-3)

### 6.4 Conclusion

Membranes prepared with a solution of density of 5%, HPS were in a continuous phase and gelatin was in a dispersed phase when HPS content was above 50%. Gelatin became a continuous phase when HPS was below 50%. The gelatin in the dispersed phase was in round shapes or olive shapes; however, HPS in the dispersed phase was in fork shapes. There were purple spots in the gelatin phase, indicating occurrence of intermingling between phases, which proved increased compatibility existed in the blends. A high blending rate and long blending time allowed more uniform dispersion of the dispersed phase in the blends, and increased intermixing between the phases, and improvement of the dispersion of blends. High membrane temperature caused HPS to form a network structure, the higher the temperature, the more compact the network structure, and the proportion of gelatin-HPS causing phase inversion changed as well. Further phase separation occurred in all blends after prolonged incubation time. HPS precipitated as the incubation time increased, consequently a small quantity of HPS existed in the upper layer of solution, the HPS dispersed in the gelatin
continuous phase as droplets. Phase separation occurred in blends of 45:55 in the shortest time among all the blends.
7. Morphologies and Phase Composition of Gelatin–Starch Blends

7.1 Introduction

Mechanical properties of starch–gelatin blends depend on their morphology, particularly the extent of homogeneity and the composition of their continuous and dispersed phases. Various techniques have been used to characterise phase composition and interface of this complex blended system by indirect methods such as differential scanning calorimetry (DSC) [42], dynamic mechanical analysis (DMA) [262, 289] and rheometry [99, 125, 129], as well as direct methods such as polarized optical microscopy and confocal laser scanning microscope (CLSM) [57]. In our previous chapter, different microscopies such as polarized optical microscopy, scanning electron microscopy (SEM) and synchrotron Fourier transform infrared spectroscopy–microscopy (Synchro-FTIRM) have been compared to study gelatin–starch blends. Of these methods, the Synchro-FTIRM provided capability to detect and measure composition and interface. Spatial resolution of FTIRM was limited to 10 x 10 μm, even application of synchrotron light technology only enabled achievement of a spatial of 5 x 5 μm [283].

Corn-starch is an economic crop that is often used for the raw materials for edible or biodegradable materials. The average diameter of corn starch granules is from 8 to 12 mm [112], so high spatial resolution is needed to examine morphology of the granules into film by gelatinization. Raman microscopy is an effective method to study heterogeneous materials since it provides sub-micron spatial resolution with high sensitivity. For example, lateral and depth resolutions of approximately 0.25 and 1.7 μm, respectively, can be achieved when using a 633 nm laser source and an aperture of 50 mm in radius to give a 60 X/1.2 numerical aperture
(NA) objective. Therefore, the aim in this study was to study morphology and phase composition of gelatin–starch blends using Raman microscopy.

7.2 Experimental

7.2.1. Materials and film preparation

Starch–gelatin blend film was prepared according to the previous chapter. Briefly, solutions were prepared with different ratios of starch:gelatin equal to 90:10, 70:30, 50:50, 30:70 and 10:90 including 1 % w/w sorbitol based on a total weight basis (2 g) in 100 mL distilled water. The mixed materials were dissolved in distilled water at 80 °C for an initial 30 min at a slow stirring speed (100 min⁻¹), then stirred for a further 30 min at high speed (700 min⁻¹) until a clear solution was obtained. The solution (5 mL) was poured onto a poly(ethylene terephthalate) (PET) dish (diameter 5 cm) that was kept level to control film thickness. The cast film was dried overnight at 37 °C. The dry films were peeled from the plate, placed in a desiccator containing saturated sodium bromide (NaBr) solution to control humidity, and stored at 56 %·RH and 23 °C until required for analysis.

7.2.2. Raman microscopy

An XploRA plus Raman confocal microscope system (Horiba scientific) was used to analyze specimen surfaces. A 532 nm diode laser (15 mW laser power) with an X100/0.90NA air objective was employed. Spatial resolution was obtained using 100 mm confocal pinholes. The Raman signal was acquired using 1200 lines/mm grating centered between 200 and 2760 cm⁻¹. A 1.7 x 1.5 μm area of the surface was mapped at X and Y-axes for each specimen. Data was analyzed using LabSpec 6. The integration time was 10 s for all measurements.
7.3 Results and discussion

Different ratios of gelatin:starch (90:10, 70:30, 50:50, 30:70 and 10:90, named G9S1, G7S3, G5S5, G3S7, G1S9) including 1% w/w sorbitol plasticizer, were used as model materials, a Raman confocal microscope system with a 532 nm laser was used to study the gelatin–starch films. Figure 7-1 shows the approach to acquisition of spectra, data analysis (image generation) and processing of the Raman images, Figure 7-1(A) shows the heterogeneous nature of such blends and their phase distribution. Gelatin was a continuous phase while starch formed a separated phase distributed as spherulites of about 5 mm diameter. It should be noted that the diameters of starch particles depend on volume fraction of starch and drying temperature. The distribution of starch was more diffuse and the interfacial contrast became less distinct depending on volume fraction and water content.

Figure 7-1(B) and Figure 7-2 show the Raman spectra of gelatin–starch and gelatin. A typical spectral feature of the pure gelatin film was the strong amide I band located at approximately 1654 cm\(^{-1}\) which usually indicates the alpha helix dominated structure. The amide I absorption
is primarily due to the stretching vibration of the C=O bond (Sun et al., 2011), and it is related to the protein conformation, such as $\alpha$-helix or $\beta$-sheet. Another characteristic peak around 1250 cm$^{-1}$ is the amide III involving C-N stretching and N-H inplane bending vibrations of the peptide bond as well as contributions from C$_\alpha$-C stretching and C=O in-plane bending. The typical starch spectra in the range 1300-800 cm$^{-1}$ was found in the gelatin–starch film, the peak 1150 (shoulder peak), 1120 and 1087 cm$^{-1}$ is due to the C-O, C-C stretching and C-OH bending. Overlapping peaks located in the region of 1173–953 cm$^{-1}$ (labelled as band 1), were the most intense bands in the Raman spectra and they were chosen as the characteristic peaks for starch; while the integrated area of the amide I band (1750-1550 cm$^{-1}$), labelled as Band 2, was used to represent gelatin. The ratio of the peak area of the starch bands (band 1) to that of gelatin bands (band 2) was used to represent the distribution of starch or gelatin in the blends. The ratio of band areas rather than absolute values was used to enable normalization of the results, correcting for possible variations in film thickness, both within the same film and between different films.

![Raman spectra of gelatin, starch and gelatin–starch blend (G3S7)](image)

Fig 7-2 Raman spectra of gelatin, starch and gelatin–starch blend (G3S7)
Raman intensity mapping of the band 1/band 2 under 532 nm laser excitation in Figure 7-1(C) shows a 2D contour map that was established for each of the blends with different gelatin-starch content. Figure 7-1(C) gives a higher special resolution to observe the phase distribution compared with FTIR microscopy, where special solution could reach 1.7 \( \mu \text{m} \times 1.5 \mu \text{m} \). The color code represents concentration of a component. The red in the scale denotes a high starch concentration while blue denotes a high gelatin concentration. The Raman maps confirmed that starch was a dispersed phase while the gelatin phase formed a continuous matrix in which starch inclusions were dispersed. The results demonstrated the heterogeneous nature of such blends with starch domains dispersed into a gelatin continuous phase, even with higher starch content blends.

Figure 7-1(D) shows overlaid data of Raman microspectroscopy–optical microscopy images. The overlaid images show that exactly the same area was mapped by Raman microspectroscopy, however Raman microscopy revealed the chemical composition. When the images were overlaid, the resultant image gave a replicate result for the distribution of starch and gelatin.
Figure 7-3 shows a chemical map of starch distribution acquired from a CCD (charge-coupled device) detector for various gelatin–starch blends. The size of starch domains decreased with increasing gelatin volume fraction. Raman concentration maps suggested that for all mixtures investigated gelatin formed a continuous matrix in which starch inclusions were dispersed. The results demonstrated the disperse and diffuse nature of such blends, with starch domains dispersed into a gelatin continuous phase, even with higher starch volume fraction blends. However, the interfacial contrast became less distinct with increasing gelatin volume fraction. During the film-forming process, the starch experiences gelatinization and gelation; over short times retrogradation of Amylose occurs, while over long times amylopectin retrogrades. Furthermore, while some Amylose will be subject to retrogradation to V-type crystals thus enhancing separation of starch as it becomes spherical[151, 152, 154], so amylose may interact...
The Raman results support the observation that starch and gelatin are compatible though not miscible, furthermore, it also provides some new results, in particular the inter-phase. More experiments on enlarging the mapping area will be studied in future.

Compared with gelatin–starch blending observed by FTIR microscopy, large spatial resolution (1.7 μm x 1.5 μm) resulted with Raman microspectroscopy. There are some intermediate phases around the starch phase that display a green color in the gelatin–starch blends. This phenomenon may be due to the amylose leached from the starch phase during gelatinization during processing, and interacting with gelatin; some sorbitol plasticizer would assist by stabilizing the interface. Intermediate phases were observed using Raman microspectroscopy, which further support the observation that starch and gelatin are compatible though not miscible.

### 7.4 Conclusion

Raman microspectroscopy mapping has been shown to be an efficient and effective method to characterize the phase composition and distribution of gelatin–starch blends. The ratio of the areas of starch and gelatin bands was used to determine the relative distributions of the two components in the blends. The Raman maps confirmed that gelatin formed a continuous matrix in which starch inclusions were dispersed, for all the blends investigated. Intermediate phases, due to amylose interaction with gelatin, were observed using Raman microspectroscopy. The gelatin–starch blends formed a compatible system with gelatin matrix and starch dispersed phase, with each component contributing to properties for application in capsules requiring enhanced gas barrier and facile dissolution to release contents.
8. Plasticizer Mitigation of Microstructure and Mechanical Properties of Gelatin–HPS Blends

8.1 Introduction

Plasticizers can greatly transform the properties of polymer film materials. Film materials free of any plasticizer may be brittle, and this is especially true for starch-based film materials that cannot become more flexible until a critical volume fraction of plasticizer is added. Plasticizers are mostly small molecules or small molecular weight polymers that can increase free volume and molecular mobility of polymers. This leads to increased mobility of macromolecules and decreased interaction among molecules, making the cellular structure of macromolecules less dense and as a result improving the ductility of film materials [293-295].

There has been much research about the impact of plasticizer type and dosage on starch [296, 297] and gelatin [298] film materials. These factors influencing the plasticizing action of plasticizers are: the glass transition of the plasticizer, the size and shape of plasticizers molecules, oxygen atoms, spatial distance of oxygen atoms, and plasticizer–water binding force. Different plasticizers have varying impact on mechanical performance and microstructure of film materials [196, 299]. Researchers have suggested addition of plasticizers may contribute to higher crystallinity of film materials [299]. Research by Zhang et al. [86] showed that poly(ethylene glycol) (PEG) as a plasticizer can improve the interfacial properties of gelatin–starch blends because PEG is compatible, though not miscible, with the two phases (solid and liquid phases).

WAXS, SAXS, UV and an extensograph were used in this research to determine the impact of three plasticizers (PEG, glycerol and propylene glycol) on the multi-dimensional
structures and apparent properties of film materials and the mechanism of plasticizer improvement of mechanical properties of these blends.

8.2 Experimental

8.2.1. Materials and film preparation

A commercially available gelatin (GELITA UG719-N, Sweden) was used in this work. A food-grade hydroxypropylated high amylose (80 %) corn starch (A939) with MS 0.11 was supplied by Penford (Australia). Poly(ethylene glycol), M=400 g/mol (PEG 400), was purchased from SigmaAldrich Chemical Co..

20 %, 15 % and 10 % starch–water solutions were initially prepared for various characterizations. Solutions were prepared with different ratios of starch–gelatin (100:0, 80:20, 60:40, 40:60, 80:20, 0:100) based on a total weight basis (100 g) in 400 mL distilled water. The materials were dissolved in distilled water at 80 °C for an initial 30 min using a slow stirring speed (100 rpm), then for a further 30 min at high speed (700 rpm) until a clear solution was obtained. 20 g of solution was poured onto a poly(ethylene terephthalate) (PET) dish (diameter 15 cm), which was kept level to control film thickness. The cast film was dried overnight at 37 °C. The dry films were peeled from the dish, placed in a desiccator containing saturated sodium bromide (NaBr) solution, and stored at 56 %·RH and 23 °C for at least one week, or until required.

8.2.2. SAXS and WAXS

Films were tested using a Philips PW 1130 wide-angle X-ray diffraction at 22 °C. A PW3830 X-ray generator with a long fine focus sealed glass X-ray tube (PANalytical) was operated at 40 kV and 50 mA. A focusing multilayer optics and a block collimator provided an intense monochromatic primary beam (Cu-Kα, λ = 0.1542 nm). The scanning speed was 50 °/min.
8.2.3. Transparency measurements

A UV (WFZ UV-3802) spectrum was used to measure the transparency of each gelatin–starch solution, which were placed in a 10 mm × 10 mm container for measurement. A wavelength of 206 nm was used to measure transparency. The transmission of different films measured at the wavelength of 206 nm was divided by film thickness (mm) and presented as %/mm.

8.2.4. Mechanical properties

Dumbbell-shaped specimens (gap 50 mm, width 1 mm) were cut from cast films, then equilibrated at 56 %·RH (humidity was regulated by NaBr solution) for 72 h before testing. The tensile properties of specimens were measured in accordance with ASTM D638 using an Instron Universal Test Instrument (Model 3366). The Young modulus, tensile strength and elongation at break were measured at a crosshead speed of 10 mm/min. Each test consisted of seven replicate measurements per film.

8.2.5. Optical Microscopy

For blends processed with PEG and without PEG, the detailed methods of preparation and staining were consistent with chapter 6.2.3; where observation of the stained membranes was performed with a Olympus BHZ-UMA microscope.

8.3 Results and Discussions

8.3.1. Analysis of Crystal Structure

Figure 8-1 is a wide angle X-ray scattering (WAXS) diagram of blends containing different plasticizers. It is found from Figs. 8-1a ~ e that different plasticizers have different impact on film crystal structure. Figure 8-1a presents the WAXS scattering pattern of pure HPS under action of different plasticizers. It is observed from the figure that there is a broad envelope peak close to a large peak at 20° for each pure HPS(0:100) according to the research by Zhang et
al.[267] and Chang et al.[300] that suggested the starch readily ages to form V-shape crystals at with diffraction at 20°. However the peak at 20° in this figure is wide and diffuse indicating that blend recrystallization was slow, possibly because the starch used was a hydroxypropyl starch, in which the substituent hydroxypropyl group sterically retarded the starch from recrystallization. Figure 8-1e shows WAXS diagram of pure gelatin containing different plasticizers.

Figure 8-1 WAXS of gelatin-HPS blends with different plasticizers, 0:100 a); 30:70 b); 50:50 c); 70:30 d); 100:0 e)

Pure gelatin (100:0) exhibited two apparent crystallization diffraction peaks at 7.8° and 20°, respectively. For blends with gelatin content less than 30%, there is a weak peak at 20°, similar to those of pure gelatin; the scattering patterns for blends containing at least 30% gelatin were consistent with that of pure gelatin: there are two diffraction peaks at 7.8° and 20°; the peak at 7.8° is the result of gelatin recrystallization while the peak at 20° is the joint result of both
gelatin and HPS crystals. Regarding blends containing the same plasticizers: it is known that at a gelatin content less than 30%, the blends recrystallize to a lesser extent compared with that of HPS, mainly because gelatin at a small content can retard HPS aging and retrogradation; when gelatin content was above 30%, the blend crystallization increased with increase in content mainly because gelatin is a linear-chain structure and the molecules interact more readily to form a crystal structure, which is inconsistent with the results of the research by Jimerse that indicated addition of gelatin could reduce HPS aging and retrogradation to a lower extent[8].

Comparison of different plasticizers at the same blend ratio revealed that, compared with unplasticized blends, glycerol had a larger contribution to the crystal structure of blends at a high HPS content, and for glycerol-plasticized film materials containing more than 70% HPS, a new crystallization peak took shape at 17°, which is a type-B characteristic peak of starch. Research by Zhang et al[267] suggested that a new type-B recrystallization peak would emerge for gelatinized starch. No new recrystallization peak formed for blends plasticized by other plasticizers: PEG and propylene glycol, compared with unplasticized blends. PEG had the most significant activity on crystallinity of blends followed by glycerol and then propylene glycol, which had the weakest action with no obvious difference from unplasticized blends. Research completed suggested that recrystallization at a higher level would take place in some film plasticized materials[301-303] partly because addition of plasticizers enhanced interaction between plasticizers and macromolecules and weakened the interaction within macromolecular phases thus preventing recrystallization, and partly because addition of plasticizers makes macromolecular chain segments more capable of segmental motions while driving the large-molecule chain segments closer to each other. This gave more opportunities for macromolecular chain segments to combine mutually to form crystals. The two mechanisms mutually contribute and under certain conditions one may prevail over another. In this system, however, the mechanism of plasticizers facilitating recrystallization prevailed.
8.3.2. Analysis of micro-region structure

Fractal dimensions \((D_m)\) of gelatin–HPS blends containing different plasticizers are shown in Table 8-2. The calculation of particle size is obtained as the fractal dimension. Inserted the calculation details as: Fractal dimension was calculated from Porod equation:

\[
I(q) = 2\pi S(d_1 - d_2)^2 \cdot q^4
\]

where \(I\) is the X-ray intensity, \(S\) is the surface area per unit volume which is the fractal dimension, \(d\) is density of two phases 1 and 2 and \(q\) is the scattering length calculated from the scattering angle using the Bragg equation. The forth power of \(q\) is fitted when the dispersed particles are spherical.

Table 8-2 shows that a similar tendency was found in fractal structures between blends, with and without PEG: with increasing gelatin content, the mass fractal dimension first decreased and then increased indicating that density of self-similar structures in film materials first decreased and then increased. This means addition of gelatin hindered mutual bonding of HPS chain segments to some extent and addition of HPS hindered mutual bonding of gelatin chain segments making for decreased density of self-similar structures. For blends containing gelatin–HPS at the same fraction, unplasticized blends have denser structures than PEG-plasticized blends, indicating that PEG impeded mutual bonding of chain segments and thus make blended self-similar structures more mobile. Blends containing glycerol did not exhibit self-similar structures. Blends plasticized with propylene glycol do not change with HPS ratio; and blends with a ratio of 50:50 are in the form of surface fractals showing the densest self-similar structures. Addition of propylene glycol increased the density of self-similar structures of all blends, except gelatin alone. In conclusion, different plasticizers showed varying activity towards the micro-region structures of film products.
Chapter 8: Plasticisers, Microstructure and Mechanical Properties

Table 8-1 Fractal structure of blends with different plasticizers

<table>
<thead>
<tr>
<th></th>
<th>0:100</th>
<th>30:70</th>
<th>50:50</th>
<th>70:30</th>
<th>100:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_m$(FC)</td>
<td>2.63±0.12</td>
<td>2.58±0.06</td>
<td>2.08±0.08</td>
<td>1.87±0.17</td>
<td>2.43±0.29</td>
</tr>
<tr>
<td>$D_m$(PEG)</td>
<td>1.57±0.02</td>
<td>1.48±0.25</td>
<td>1.16±0.09</td>
<td>0.90±0.07</td>
<td>1.04±0.01</td>
</tr>
<tr>
<td>$D_m$(Glycerol)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$D_m$(Propylene glycol)</td>
<td>2.71±0.16</td>
<td>2.58±0.26</td>
<td>—</td>
<td>2.17±0.07</td>
<td>1.83±0.27</td>
</tr>
<tr>
<td>$D_m$(Propylene glycol)</td>
<td>—</td>
<td>—</td>
<td>2.79±0.08</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Figure 8-2a-e shows the small-angle X-ray scattering diagram of films containing different plasticizers. No diffraction peaks occurred within the range 0 ~ 0.03 nm for all unplasticized blends and those plasticized with PEG and propylene glycol and a peak occurs at 0.004 nm only for blends plasticized with glycerol, which is related to a structure of which the distance between half-crystallized crystal faces is 15.7 nm. No obvious peak was found at 0.004 nm for another series of glycerol-plasticized blends kept at a low relative humidity (51%). The possible reason is that at high RH, high film moisture content facilitated chain segmental movement, with interaction and bonding mutually generating supramolecular structures, similar to the process of forming crystal structures. It is concluded that both moisture and glycerol are involved in formation of such part-crystallized lamellar structures. It was observed that within a small angle ($q<0.2$ Å), among blends having the same blend ratio, PEG-plasticized blends had the largest scattering strength, followed by glycerol, and no significant difference was found between propyleneglycol–plasticized blends and those unplasticized. This infers that the difference in electron density is greatest between the amorphous region and the crystal
Chapter 8: Plasticisers, Microstructure and Mechanical Properties

micro-region for PEG-plasticized blends. Though glycerol-plasticized blends have the largest crystallinity, glycerol contributes to the mobility of blend amorphous regions not as significantly as PEG. That is why PEG-plasticized blends had the largest difference in electron density. The previous wide-angle X-ray analysis revealed that propylene-glycol–plasticized blends had a higher crystallinity, though not as high when compared with unplasticized blends. Furthermore, research by some scholars[196] demonstrated that propylene glycol is not an effective plasticizer and has poorer ability to increase mobility of HPS or gelatin amorphous chain segments. As a result, the difference in electron density between crystal micro-regions and amorphous regions was not as significant for propylene glycol–plasticized blends as that for PEG or glycerol–plasticized ones. It was found from the amplified picture that peaks occurred at 0.04 nm and 0.056 nm respectively, consistent with the peak value of type-B crystallization of HPS at 5.6° (type-B crystal of HPS) and of the crystallization of gelatin at 7.8° (gelatin crystal). The peak area at 0.04 nm and 0.056 nm decreased with decreasing content of HPS and gelatin respectively, mainly because with increasing ratio of relevant constituents they have more opportunities to bond mutually resulting in increased crystallinity. The crystallization peak area was the largest at 5.6° and 7.8° for glycerol-plasticized blends, by comparing blends at the same blend ratio, the result was consistent with that from the wide-angle X-ray scattering analysis. The peak at 5.6°, was not observed in the wide-angle X-ray scattering because it was very weak peak and may have been concealed under the broad background scattering.
8.3.3. Analysis of transparency measurements

Figure 8-3 shows the action of different plasticizers on light transmittance of films. It was found that with increasing HPS content, the light transmittance decreased first and then increased, indicating that the blends were compatible, though not miscible and phase-separation took place. For pure films (0:100, 100:0), addition of plasticizers lead to decreased light transmittance mainly because plasticized blends have higher crystallinity. The reduced light transmittance resulted from crystallization that facilitated light refraction and reflection. For blends having the same ratio, all three plasticizers increased the light transmittance of films mainly because the plasticizers decreased inhomogeneous structures at the interface of film materials and the improvement overcame that of structures reducing light transmittance due to increased crystallinity of blends. Among all plasticizers, glycerol was the most active in increasing light transmittance of blends, indicating that it minimised inhomogeneous structures at the interface between two phases (solid and liquid) leaving the interface between...
the two phases interdiffused. Research by Zhang [86] suggested that addition of plasticizers improved the interfacial structure of gelatin–starch blends. Both PEG and propylene glycol had similar function of increasing light transmittance of films, but their action was not as significant as glycerol, propylene glycol having the poorest activity.

![Figure 8-3Plasticizer activity on the transparency of gelatin–HPS blends](image)

**8.3.4. Analysis of mechanical properties**

Mechanical properties of blends containing different plasticizers are shown in Table 7-5. Plasticizers created large changes to the mechanical properties of the film materials. There was no significant difference in the tensile strength and modulus between pure gelatin and unplasticized HPS. After addition of plasticizers, both the tensile strength and modulus reduced significantly and elongation at break increased significantly. Film tensile strength and modulus decreased even more with increasing HPS content. PEG had a better plasticizing activity than glycerol for blends and pure gelatin; while glycerol was better for pure HPS. Possibly because HPS contains more free hydroxyls that allow formation of intermolecular hydrogen linkages with glycerol, and blends containing glycerol had strongest hygroscopicity, they absorbed more moisture [305, 206], while pure HPS was the most sensitive to moisture, glycerol gave the most
significant plasticization to pure HPS. PEG contains more ether linkages allowing it to interact with gelatin more strongly, because it has a high degree of etherification linkages. Propylene glycol–plasticized blends have the smallest tensile strength, modulus and elongation at break indicating propylene glycol had the weakest plasticizing activity. This is consistent with the result of research by Sloan [304].

![Graphs showing tensile strength, elongation, and modulus for GEL-HPS blends with different plasticizers and concentrations.](image-url)
Previous researchers suggested that, in terms of the level of significance at which plasticizers increase polymer crystallinity, the trend is glycerol > PEG > propylene glycol > no plasticizer. Plasticizer activity in improving the density of micro-region structures of blends is correlated with chemical structure; in terms of the degree to which plasticizers mobilise amorphous region structures the trend is PEG > glycerol > propylene glycol > no plasticizer. However, from Figure 8-4, the activity with which plasticizers improve mechanical properties of blend blends the trend is PEG > glycerol > propylene glycol. In conclusion the activity of plasticizers on mechanical properties of blends depends upon increased segmental mobility of the amorphous region of blends.

It is found that tensile strength, mechanical properties and modulus of un-plasticized blends did not change with increasing gelatin content, indicating a significant phase separation occurred. Tensile strength and modulus increased with increasing gelatin content for plasticized blends, and elongation at break decreased gradually with increasing gelatin content. This indicated blend compatibility was improved and therefore plasticizers had a secondary action as compatibilisers. Research by Zhang et al.[86] demonstrated that PEG can be used as a plasticizer and a compatibilising agent in gelatin–HPS blends to diffuse the interface between the two phases and to facilitate mutual bonding of two different materials making the two phases more compatible.

8.3.5. Analysis of optical microscopy

Fig 8-5 shows the morphologies of the starch (50)–gelatin (50) blends with and without PEG, observed under optical microscopy. In order to enhance the phase contrast, the starch was stained with iodine, which increased darkness of starch phase. The results demonstrated the highly heterogeneous nature of such blends and their phase separation. It is seen that generally
gelatin is a continuous phase while starch is a separated phase distributed as particles of about 80 µm diameters in gelatin. It has been noted that the distribution of starch is more homogeneous and the interfacial details became dimmer even at the edge of particles that were more identifiable after adding PEG, indicating that compatibility between gelatin and starch was improved.

Figure 8-5 The blends of gelatin (50)–starch (50) without (a) and with 5 % PEG (b) observed under optical microscope (the starch was stained by iodine)

8.4 Conclusion

Optical microscopy demonstrated the highly heterogeneous nature of such blends and their phase separation. The results showed that generally gelatin was a continuous phase while starch was a separated phase, distributed as spherulites of about 80 µm diameter in gelatin. This research has investigated plasticizers activity on multiple-dimensional structures and properties, and interpreted the mechanism of plasticizer action on mechanical properties. The level of significance at which plasticizers increased crystallinity was glycerol > PEG > propylene glycol > no plasticizer. The impact of plasticizer on micro-region structures of blends is correlated with chemical type: PEG reduced the density of self-similar structures of blends; propylene glycol increased the density of self-similar structures of all blends, except pure gelatin; glycerol–plasticized blends did not have self-similar structures but had 15.7 nm part-crystallized structures. Plasticizers improved mechanical properties of blends in the order PEG > glycerol > propylene glycol. The plasticizer activity on mechanical properties of
blends depended on plasticizer increasing mobility of the amorphous region of blends. However, something special occurred for pure HPS blends; glycerol improved their mechanical properties the most, which was caused by high moisture content in pure starch that was plasticized with glycerol. After plasticizers addition, blend tensile strength showed a unitary trend to change with increasing HPS content; and plasticizers improved light transmittance, indicating all of these three plasticizers improved gelatin–HPS phase compatibility.
9. Conclusion and Proposal for Further Research

9.1 Conclusion

The impact of concentration, gelatin–starch blend ratio and temperature on blends viscosity and gel structure has been investigated; and the impact of different heat-treatment conditions (i.e. cooling rate and drying temperature) on blend micro-structure and performance. Further, the relation between gel structure, film structure and film properties under certain heat-treatment conditions is discussed.

1. Drying temperature impacted on the gel structure of the blends while they were dried, then the crystal structure and micro-region structure of film materials, and finally their mechanical properties varied significantly. For blends with the same gelatin–HPS ratio, gel-dominated the gelatin network at high temperature and it had higher strength and density than its HPS-dominated counterpart at low temperature, resulting in increased high-temperature crystallinity and denser amorphous regions for film materials. Increased crystallinity and structures having denser amorphous regions jointly contributed to increased film tensile strength and modulus at elevated temperature.

2. The cooling rate, though posing no significant impact on blend crystal structures, impacted on the density of the self-similar structure of micro-regions of film materials, and in the plasticised blend systems. The density of the self-similar structure of the film material significantly influenced tensile strength and modulus. For blends containing HPS higher than 50%, the gel structure of blends that were cooled at a slow rate developed high regularity and density, leading to increased density of micro-region self-similar structure and finally higher tensile strength and modulus for these blends. When HPS content was below 50%, for the blends that were cooled at a fast rate, the long-molecular chains bonded mutually to a
greater degree, this made for higher density of micro-region self-similar structure and finally increased modulus and tensile strength for the film materials that were cooled at a fast rate.

The impact of processing factors on blend phase changes and compatibility was investigated by applying optical microscopy with an iodine visualisation method. FTIR spectroscopy and scanning electron microscopy were used to further study system compatibility, and in consideration of the observations from microscopy, the phase change and compatibility was analyzed and investigated comprehensively. Use of ultraviolet spectroscopy, contact angle measurement and extensography to measure light transmittance, contact angle and tensile properties, system compatibility, phase change and film properties were evaluated.

For films made from solutions at a concentration of 5%, when HPS content was higher than 50%, HPS was a continuous phase and when concentration was lower than 50% gelatin was a continuous phase; therefore HPS content of 50% was the composition for phase change. The film gelatin–HPS ratio was changed by solution temperature, which caused a phase change and impacted on the system phase distribution pattern. High stirring rate and longer time of agitation helped to make the dispersed phase disperse more evenly and generate more inter-phase blending in the system, thereby improving its compatibility. An increase in the duration of blends being held statically caused more phase separation for all blends. Settling of HPS finally left a small amount of HPS remaining as a residue in the upper solution, to be dispersed in the form of very small drops into the gelatin continuous phase. Among all gelatin–HPS blends, phase separation took place over the shortest time with a 45:55 composition.

The relation between compatibility, phase change and film material properties was compared. FTIR and SEM observation indicated that the blend system was semi-compatible. It was observed from optical microscopy that phase change occurred at HPS content of 50%. The optical transmittance of the blended film material increased with increase in HPS content, but the trend was reversed at an HPS content of 70%, which mainly resulted from the system phase
change and compatibility. There was a turning composition for the modulus at an HPS content of 50%, while the contact angle positively deviated from linearity, connecting pure films at an HPS content of 50% and negatively deviated from linearity at an HPS content of 50%, at which a phase change occurred in the system with HPS content of 50%.

3. The condition of plasticizer or crosslinking agent, and the relation between the micro-structure and performance of film materials was investigated.

This research systemically investigated the action of plasticizers on multiple-dimensional structures and properties of gelatin–starch blend systems and interpreted the mechanism of plasticization and improvement of mechanical properties. The level of significance at which plasticizers increased crystallinity is: glycerol > PEG > propylene glycol > no plasticizer. The impact of plasticizer on micro-region structures was related to plasticizer structure: PEG reduced the density of self-similar structures; propylene glycol increased the density of self-similar structures of all blends; though not of pure gelatin; glycerol–plasticized blends do not have self-similar structures, they have 15.7 nm partly-crystallized structures. Plasticizers reduced vitrification in the order: PEG > glycerol > propylene glycol and from this it is inferred that, plasticizers lubricated the amorphous region structures in the order: PEG > glycerol > propylene glycol > no plasticizer. Plasticizers improved mechanical properties of blends in the order: PEG > glycerol > propylene glycol. It is concluded that the action of plasticizers on mechanical properties of blend films depended on plasticizer selective increase of amorphous region mobility of the gelatin–starch blends. However, something special occurred in pure HPS film. Glycerol significantly improved mechanical properties of blend films, which was related to the high moisture content of pure starch that was plasticized with glycerol. After plasticizers were added, tensile strength showed a unitary trend to change with increasing HPS content; and plasticizers improved light transmittance, indicating that the three plasticizers each contributed to blend compatibility improvement.
9.2 Innovation

1) Gelatin–starch blends were investigated systematically, with measurement of properties such as modulus, self-similar-structure, density and aggregate gel dimensions. These properties were dominated by different fractions in the gelatin–HPS reverse-phase gel blends and they determined the relationship between gel structures, film material structures and film material performance.

2) Iodine solution was applied to selectively visualize HPS in gelatin–HPS blend films and created a method that allowed observation of phase distribution and phase changes in gelatin–HPS blends using optical microscopy, which is methodologically instructive and instrumental in quantifying phase distribution of other starch-based blends.

3) The mechanism of plasticizer action was investigated in the multiple-dimensional structures and properties of blend film materials. This brought forth a mechanism of plasticizer improvement of mechanical properties.

9.3 Proposed further research

The results of this PhD thesis have shown that plasticizers such as PEG and glycerol do not necessarily avoid the phase separation that affects the rheological, processing and mechanical properties of the gelatin–starch blends. During the course of this PhD research, cast films that were unaffected by solution phase separation proved to be quite challenging, due to technical problems encountered and limitations of the equipment. Therefore, plans for future work must address these limitations and undertake a comprehensive study to evaluate the specific conditions of phase separation that can extenuate the difficulty of preparing films without affecting the bulk properties.
In addition, data exists to explain the modification of starch or gelatin by cations; no reports are available on the effect of salts on starch–gelatin interactions. Future work should involve a study to explore cation actions on gelatin–starch blend phase separation. This could be a viable approach to improving the bulk properties of gelatin–starch blends. Controlling the phase separation of the blends by plasticizers or compatibilizing agents can lead to further enhancements in mechanical and thermal performance of the final composites, which can potentially result in new applications for these composite materials.

The key to developing gelatin–starch blends for more engineering applications is to find the correct balance between strength and toughness. One possible approach is selecting HPS with different degrees of hydroxypropyl substitution or starch from different sources. Other potential factors could be the source of gelatin, including beef skin or fish skin. Any research involving different starch and gelatin will require careful planning and correct processing to reduce influence of shear, temperature and pressure. The action of cross-linkers could be explored to evaluate their influence on processing, mechanical properties and thermal performance.
10. References


[16] Lin D., Zhao Y. Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables . Comprehensive Reviews in Food Science and Food Safety, 2007, 6(3): 60-75


References

Trends in Food Science and Technology, 2003, 14: 362-373

[22] Singthong J., Thongkaew C. Using hydrocolloids to decrease absorption in banana chips. 
LWT- Food Science and Technology, 2009, 42: 1199-1203

[25] Li X., Jiang F., Ni X. Preparation and characterization of konjac glucomannan and ethyl cellulose blend films. 
Food Hydrocolloids, 2015, 44: 229-236

Food Technology, 1986, 40(12): 47-59

Food Science, 1989, 54(6): 1393-1399

[31] Chen M.C., Yeh H.C., Chiag G.B. Antimicrobial and physicochemical and properties of methylcellulose and chitosan films containing a preservative. 

[38] Dogan N., Mchugh T.H. Effects of microcrystalline cellulose on functional properties of hydroxyl propyl methyl cellulose microcomposite films. 

Food Research International, 2008, 10: 400-405

[40] Cheng L.H., Karim A.A., Seow C.C. Characterisation of composite films made of konjac glucomannan (KGM), carboxymethyl cellulose (CMC) and lipid. 


[48] Li X., Qiu C., Ji N. et al. Mechanical, barrier and morphological properties of starch nanocrystals-reinforced pea starch films. Carbohydrate Polymers, 2015, 121: 153-162


References


[147] Chao S.J., Lai L.S. Effect of salt on the gelling behavior of hsian-tsao (Mesona procumbens Hemsl) leaf gum as studied using rapid-visco analyzer. Shipin Kexue (Taipei), 1999, 26: 228-239


References


[235] Cao N., Fu Y., He J. Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. Food Hydrocolloids, 2007, 21: 573-584


[286] Sammon C., Bajwa G., Timmins P., et al. The application of attenuated total reflectance Fourier transform infrared spectroscopy to monitor the concentration and state of water in solutions of a thermally responsive cellulose ether during gelation. Polymer, 2006, 47: 577-584


[300] Chang F.D., He X.W., Huang Q. The physicochemical properties of swelled maize starch granules complexed with lauric acid. Food Hydrocolloids, 2013, 32: 363-372


